THE YEASTS

THE YEASTS

BY

ALEXANDRE GUILLIERMOND, D.Sc.

PROFESSOR OF BOTANY, UNIVERSITY OF PARIS

TRANSLATED AND THOROUGHLY REVISED
IN COLLABORATION
WITH THE ORIGINAL AUTHOR

BY

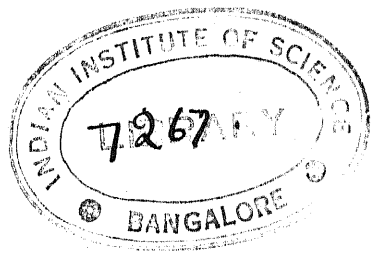
FRED WILBUR TANNER, M.S., PH.D,

PROFESSOR AND HEAD OF DEPARTMENT OF BACTERIOLOGY,
UNIVERSITY OF ILLINOIS

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FOREWORD

NO class of microöiganisms has been more intimately associated with the progress and development of the human race than the yeasts. Since the earliest times, these microörganisms have been used to bring about changes which it would have been difficult to have accomplished by other methods. Since microscopic examinations have revealed the presence of yeast cells in bread found with Egyptian mummies, it is known that these people were familiar with yeast fermentations, although they probably did not have explanations for the changes which were observed. The Norsemen prepared an alcoholic drink from milk, as is done today by certain nomadic races, the fermentation of which was, in part, caused by yeasts. Today we find the yeasts of ever-increasing interest and importance. The food microbiologist must understand the physiology of these organisms if he is to successfully cope with them. They are assuming greater importance in medicine, especially in relation to certain deficiency diseases, constipation, and skin infections. Great industries have been established which rest entirely on the chemical changes brought about by yeasts and their enzymes; some of them would be developed with difficulty, were it necessary to use strictly chemical methods. The compressed yeast industry itself has reached a high state of development with its several factories located in different parts of America and distributing agencies in practically all of the cities and villages. Many industries have been greatly changed by the availability of fresh, active yeast whenever it is needed. Despite the facts that yeasts have always been of great significance to the human race and that they will probably have greater significance in the future, it remained for Guilliermond to collect the various data, which have accumulated in regard to them, into one volume. Several treatises have been prepared which deal with the yeasts in relation to fermentations, but no real definitive treatise on the yeasts, as such, has appeared which is comparable to the volume prepared by Guilliermond. The investigations of this authority make the book especially valuable. These facts made it seem advisable to translate the volume for publication in the English language in order that the data might be available to the practitioners and students who do not read

the French language. It is sincerely hoped, however, that the rendition of this book into the English language will in no way inhibit the study of the French language — that language in which Pasteur, Bernard, Magendie, Berthelot, and others have published their classic investigations.

This English edition is based on Guilliermond's "Les Levures," which was published in 1912, appearing as a volume in the section on Cryptogamic Botany of Encyclopédie Scientifique. This series is edited under the direction of Doctor Toulouse. To merely translate a volume on a subject which is being developed as rapidly as the yeasts would be entirely inadequate. Consequently with the collaboration of Professor Guilliermond, the translator has added much new material which has been published since 1912. Without the assistance of Professor Guilliermond, this could not have been done as completely. The English edition may not, then, be regarded as a mere translation of the last French edition.

The rendition of a text from a foreign language into the English language is beset with difficulties which are most clearly appreciated by those who have performed similar pieces of work. In all cases a literal translation has not been attempted; however, the opinions of the original author have been given as closely as possible. It is trusted that this English edition will make it easier for students to pursue their study of these important microörganisms and for the practitioner to more easily solve the problems with which he has to cope. I owe many thanks to my colleagues, too numerous to mention here, for expression of their advice at various times and for their interest during the progress of the work.

University of Illinois
Urbana, Illinois
June, 1919

Fred W. Tanner

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AUTHOR'S PREFACE

SINCE the celebrated memoir by Pasteur on alcoholic fermentation, the yeasts have never ceased to assume an ever-increasing importance in agriculture and the industries. The classic investigations by Pasteur, followed by those of Hansen, have shown the profit that may result from a methodical study of the various species of yeasts, by a knowledge of the conditions necessary for their development and biochemical characteristics for application to the fermentation industries. No one may overlook the benefits which came to such industries by the use of pure cultures and selected species, and the avoidance of yeasts which caused defects in fermented products. The fermentologists have also benefited greatly by these methods. Finally, the relatively recent investigations have shown the relationship of yeasts to certain diseases in man and animals.

From a purely theoretical point of view the yeasts, on account of the facility with which they allow themselves to be cultivated in artificial media, and by the relatively large size of their cells, are especially favorable objects for experimentation upon which very important investigations of physiology, cytology and sexuality have been made. They have contributed appreciably to the progress of general physiology and biology.

It seemed useful to me to collect into one book all of the knowledge required on the morphology, physiology and taxonomy group of fungi, and to arrange it in such a manner that the data would be available for biologists, practitioners in industrial work, agriculturalists and physicians. That is what I attempted to accomplish in the little volume published in the *Encyclopédie Scientifique* under the editorial supervision of Dr. Toulouse.

Professor Tanner, of the University of Illinois, undertook the translation of this book into the English language in order to render it more accessible to American students and American investigators. This is indeed a great honor to me, one which I did not dream of when I prepared this modest work a few years ago. I am very happy to have this indication of friendship between scientific America and France, a friendship which I hope may become stronger and stronger. One sufficiently understands the significance of a scientific alliance of

two nations which by their individual characteristics supplement each other. France claims such great teachers as Lamarck, Claude Bernard, and Pasteur, true pioneers in the field of biology. By her spirit perhaps, too traditionalistic and suppressed by old administrative machinery, she has not always understood fully the real utility of her universities and given to her scientists the necessary means for carrying on their work. On the other hand, America, with no such rich heritage from the past, has built up modern laboratories with a new spirit and equipped them with the necessary resources. She probably possesses the greatest universities in the world. She lays claim to able investigators and, thanks to her marvelous scientific organization and to her numerous investigators, is sure to gain very rapidly a foremost place in the scientific world. If the American savants have the desire to profit by the discoveries of the French, their elders, France has much to gain by imitating America in her practical ideas, her spirit of organization, her methods of work, and her tremendous activity.

The book which Professor Tanner has undertaken to present to the public cannot be regarded as a simple translation of my work; it is a new edition resulting from intimate collaboration of translator and author. Microbiology is progressing so rapidly that the French edition, now six years old, is no longer abreast with recent acquisitions of the science. It was found necessary to make numerous editions and to modify certain chapters in which Professor Tanner and myself have shared the labor. Professor Tanner, known by his work on the biochemistry of bacteria, has undertaken the revision of the Chapter on Physiology of the Yeasts which was no small task, for since the discovery of zymase by Buchner, the biochemical investigations on yeasts have followed each other without interruption and have become increasingly valuable. As for myself, I have borne the task of revising the Chapters on Morphology, Phylogeny and Description of Species, subjects with which I am more familiar. Professor Tanner had, then, a preponderant part in the translation of this new edition and the book has certainly gained much by the collaboration of a physiologist so well qualified.

ALEXANDRE GUILLIERMOND

LYON, *September* 8, 1919

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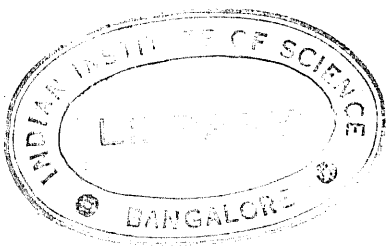
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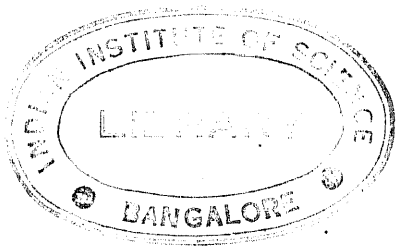
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INTRODUCTION

What are Yeasts?

UNDER the name of yeasts have been generally grouped all microorganisms which, when placed in sugar solutions, decompose them into alcohol and carbon dioxide—cause alcoholic fermentation. Knowledge with regard to the chemical properties of the yeasts has, to a great extent, preceded that with regard to their nature. The old word yeasts (Fr. *levure* = Latin *lever*) which emphasized their chemical properties dates from an epoch when no attention was given to their biological significance or nature. But today the name yeast has taken on a restricted meaning among botanists. In the botanical sense, yeasts are unicellular fungi of biochemical interest, spherical or oval in shape, and which multiply by budding. A yeast, then, is a fungus with special morphology. Be that as it may, the term is not applied to an indefinite group of fungi but to a natural one.

Many fungi, more or less developed, living normally with a mycelium are able to reproduce by budding of their filaments, to form cells which have the shapes of yeasts. These multiply in their turn by budding and retain the form of yeasts for many generations. (Fig. 1.) The basidiospores of certain *Basidiomycetes* (*Calcera viscosa*) and ascospores of certain *Ascomycetes* (*Sphaerulina intermixta Taphria*) give rise to yeasts and it is only after living for a certain time in this form that the yeast cells elongate filaments and produce a mycelium. Among the *Ustilaginales*, the sporidia, which spring from the promycelium, exist also in the shape of yeasts; it is this state in which they develop, and which they constantly retain when cultivated in artificial media. The Mucors, when placed in sugar solutions, are able to dissociate their filaments into round bodies, or buds, in a similar manner as the yeasts. *Dematium pullulans* (Fig. 1), a mold with a well-differentiated mycelium, produces in a regular fashion, by budding of its filaments, numerous yeast conidia; when these are cultivated under certain conditions, they are transformed with difficulty into mycelium. Vegetation with forms like yeasts is, then, rather widespread among the fungi.

Aside from these fungi, in which yeast forms are merely stages of development, there are others which live constantly in the forms of

yeasts. These do not present a true mycelium at any time. They reproduce at times by elongation of their cells, which adhere together, forming structures resembling mycelium; but these never offer the complexity of a typical mycelium. In the category of yeasts belong the alcoholic ferments and all of the fungi more generally known under the name of yeasts.

These yeasts, which are often designated as "true yeasts" in contradistinction to "yeast-like fungi" derived from more highly developed fungi, are not distinguishable in any manner from the latter. The general form and the internal characteristics of the cells are the same in both cases. Physiologically, certain true yeasts differ only from yeast forms of molds by their resistance to anaerobic conditions and exceptional activity of the fermenting function, but very many yeast-like structures, derived from fungi more highly developed, are equally capable of producing alcoholic fermentation, and only differ, from this point of view, from true yeasts by a decreased activity of fermentation. On the other hand, a certain number of true yeasts are totally deprived of the fermenting function. It is understood then, how the early investigators were much confused when it became necessary to characterize the yeasts.

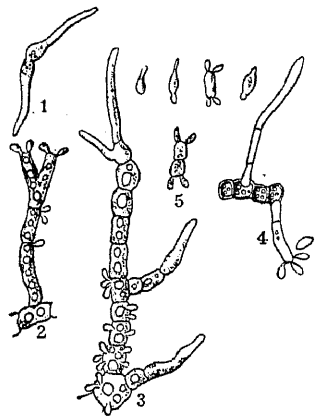


Fig. 1. — *Dematium pullulans.*
1 to 4, Mycelium Forming Yeasts; 5, Yeast-Like Bodies in Process of Budding (after Loew).

In the meantime, an essential difference which did not escape investigators existed between the yeast-like fungi and the yeasts properly so-called. Indeed, most of the true yeasts are distinguished closely from "yeast-forms" by their aptitude to produce resistant endospores at certain stages in their life cycles (unfavorable conditions), in the interior of their cells; the cells are then transformed into sporangia. De Bary, Rees, and Hansen first compared these sporangia to asces of *Ascomycetes*, considering the true yeasts as autonomous fungi which live only in the form of yeasts and are incapable of developing a mycelium.

This conception is definitely admitted today, as we shall see when the origin and systematic relationships of the yeasts are taken up. The autonomy of the yeasts and their incorporation as a group of *Ascomycetes* have been demonstrated only since Hansen observed their life cycles in nature and since certain investigators have given evidence in the origin of the asc of certain yeasts, of the presence of

a sexuality quite comparable to that which is observed in the lower *Ascomycetes*. The yeasts make up a family of the *Ascomycetes* known as *Saccharomycetes*.

True yeasts never produce endo- or ascospores. Do they represent forms derived from more highly developed fungi and made constant by a long adaptation to this condition? Or, are they true Saccharomycetes having lost, by a series of unknown circumstances, their aptitude of forming spores? We shall see that a definite loss of this characteristic has often been proved among the Saccharomycetes during certain special conditions. Be that as it may, the origin of the yeasts is entirely ignored; the Saccharomycetes are then separated and regarded as yeasts of uncertain origin. In this book, we shall examine extendedly only the true yeasts; first, yeasts with ascospores, or Saccharomycetes; secondly, those cells which exhibit all the characteristics of Saccharomycetes with the exception of ascospore formation and which, from the above, one might call pseudo-yeasts. All of those yeast-like structures of other fungi will be neglected. True yeasts are very abundant in nature; over five hundred are known. The limits of our study must be rather wide.

History of the Study of Yeasts

The study of yeasts is intimately associated with that of fermentation. The idea that alcoholic fermentations are caused by living organisms originated with Linné. In 1680 Leewenhoek first described the yeasts. He described them as globular bodies, oval or spherical in shape. In 1799 Fabroni compared yeasts to albuminoids. About 1825, and for some time after this, Mitscherlick, Cagnard-Latour, Schwann, and Kützing demonstrated that beer and wine yeasts were composed of cells which multiplied by budding. In 1839 Schwann observed, for the first time, endospores in yeasts. He proved that they might be freed by a rupturing of the cell wall.

But the nature of yeasts has been definitely known since the period in which Pasteur commenced his investigations on fermentation. Up to this time, it was known that beer yeast multiplied when introduced into saccharine wort; it was believed that it was formed spontaneously and that, in the yeast, was an occult force which produced the fermentation; that was all there was to it. With Pasteur, definite knowledge with regard to yeasts commenced. It was in 1859 that he established, by his memorable experiments which cannot be reproduced here on account of the lack of space, that fermentation is correlative with the life of yeasts. Some years later, he demonstrated the impossibility of spontaneous generation

and introduced the methods of pure culture which permitted a morphological study of yeasts.

After this, the yeasts were subjected to many investigations. In the meantime, the methods of culture were not quickly taken up, and their perfection was rather slow. It was also difficult for the earlier investigators to distinguish between yeasts and other microörganisms which developed at the same temperature. The early work on yeasts conflicted with the erroneous conceptions of the pleomorphists who maintained that the microörganisms could be reduced to a small number of species capable of exhibiting different shapes dependent upon the conditions. About 1871, Bechamp reported that the acetic acid bacteria could change into yeasts. In 1872, Trecul thought that he had obtained the transformation of the spores of *Penicillium glaucum* into yeasts. In 1875, Robin stated that many of the yeasts (*Torula cerevisiae*, *Mycoderma cerevisiae*) are, with *Penicillium glaucum*, only forms of the same fungus. A little later, however, very careful investigations were reported. Between 1868 and 1870, Rees observed endospores in many species of yeasts, and gave the first accurate description of these organs. This was followed by the work of Engel, Seynes, Brefeld, and de Bary. The last of these, in his "Morphology and Biology of the Fungi," classed the yeasts among the *Ascomycetes*.

The introduction and perfection of pure cultures permitted the exact morphological studies of the yeasts. This was the work of Hansen, who is the true founder of this study, and whose name marks a second step in the history of the yeasts. Through his careful investigations for a period of 30 years, this mycologist perfected methods which were introduced by Pasteur for culturing and isolating the yeasts. He succeeded in inoculating cultures with a single cell and separating one species from another. By careful studies on the morphological and physiological properties of yeasts, Hansen found the characteristics which allowed the differentiation of one species from another. He has thus been able to characterize a large number of species the majority of which are known. Hansen is responsible for our knowledge of the life cycle and the systematic relationships of the yeasts. In recent years, he has proposed a classification which has been universally accepted.

The third step in the study of yeasts was the discovery, by Buchner, of zymase, which allowed a considerable advance in the study of yeast nutrition and the mechanism of alcoholic fermentation. Thus, as has been said, three names, Pasteur, Hansen and Buchner, remain intimately associated with the study of the yeasts and will constitute the pivot about which our investigations will center.

BANGALORE

PART I — GENERAL

CHAPTER I

MORPHOLOGY AND DEVELOPMENT OF YEASTS

General Characteristics of Yeasts. The Different Phases in Their Development

THE yeasts are unicellular fungi; generally, they live in isolated states. After they have acquired a certain size, they divide and produce daughter cells which are not slow to separate, enlarge, and divide in their turn in the same manner.

In almost all of the yeasts, cellular division takes place by budding or gemmation. There are a few species from warm climates in which multiplication is effected by transverse division. By reason of this particular method of division, these species have been brought together into a special group and named *Schizosaccharomycetaceae.*

Budding consists in the appearance, on any side of the cell, of a little bud which slowly increases in size until it ultimately becomes a cell identical with the mother cell.

Partition simply consists of the formation in the middle of the cell of a wall which divides the cell into two daughter cells of equal size which grow eventually.

When division of the cell takes place rapidly, it often happens that many buds are formed simultaneously at different points on the surface, and these daughter cells begin to multiply before separating. This forms a little colony, or conglomeration, of cells. The same phenomenon is observed in the case of the *Schizosaccharomycetaceae.*

In old cultures and under certain conditions, the cells remain united in long chains; this gives the appearance of a mycelium, but it always remains in a rudimentary state.

The yeasts are then able to present two forms: one, which is most frequent, represents the normal type of vegetation. In this the cells are isolated or united in little groups; the other, which is quite exceptional, is the filamentous or mycelial state.

When the yeast finds, in the medium in which it is cultivated, favorable conditions for growth, it divides actively until the conditions become unfavorable from the lack of food or the accumulation of

products of metabolism; it ceases, then, to divide and produces organisms which allow it to perpetuate itself over unfavorable conditions.

Will and Casagrandi, under these conditions, have observed cells filled with reserve products (fats and glycogen) enclosed in a thick wall. They offer a great resistance thanks to these reserve products, which they retain for a long time during suspended activity, until favorable conditions allow them to develop again. The cells, which are comparable to cysts, have been designated under the name of "durable cells."

But the ordinary process employed by the yeasts for perpetuation of the species is sporulation; a certain number of internal, or endospores, are formed in the interior of each cell. The cells are thus transformed into a sort of sporangium which is called an asc. The spores or ascospores formed in these ascs are endowed with a great resistance against external conditions and during years of suspended activity. When placed in favorable conditions, they swell up and rupture the asc wall to become free. They then offer the appearance of vegetative cells and multiply in the ordinary way.

In certain species, the formation of the asc is preceded by a sexual process; the asc then results from the fusion of two cells — a copulation as in the case of an egg. In other species, sexuality is maintained in a lower state of development; in this case, it takes place between two spores at the moment of germination. In the greater number of yeasts, however, no sexuality has been observed.

Many of the yeasts, as *Torula* and *Mycoderma*, do not form endospores. We shall investigate successively, in this chapter, the morphological characteristics of yeasts: the form and shape of the cells, mycelial formations, durable cells, cellular division, formation of the asc, sexuality, and germination of ascospores.

Forms of Cells

The yeasts offer forms varying usually from a sphere to an ellipse. They possess quite a thick membrane. The greater number of them have a colorless interior containing vacuoles and refractive granules. Often, a red pigment may be observed, sometimes a brown, gray or yellow one; in this case it is probably not a true *Saccharomyces* but a yeast without endospores. However, Hansen [1] has observed a rose-colored yeast which did produce endospores. The dimension of yeast cells varies between from 1 to 4 or 5 μ in width and from 1 to 5 or 9 μ in length. There is a great difference in the cells of the same species. The yeasts are very polymorphic and are capable of assuming

[1] Hansen, E. C. 1879. Comp. Rend. des trav. du lab. de Carlsberg, 24.

different forms, depending upon the medium in which they are cultivated and their age. It is thus, for example, in old cultures that the wider cells diminish generally at the expense of the longer ones. The different species of yeasts have somewhat the same shape and are distinguished with some difficulty from one another. If *S. cerevisiae* is compared with *S. Pastorianus* or *S. ellipsoideus*, quite noticeable differences are apparent. While *S. cerevisiae* usually presents round cells and *S. ellipsoideus* egg-shaped cells, *S. Pastorianus* presents, to the contrary, elongated cells, often in the shape of a sausage. But besides these elongated forms, one may find in cultures round cells which may scarcely be differentiated from *S. cerevisiae* or *S. ellipsoideus*. On the other hand, in culture of *S. cerevisiae* and *S. ellipsoideus* may be found round cells, and also elliptical cells which bear much resemblance to *S. Pastorianus*.



Fig. 2. — *Saccharomyces cerevisiae* (after Hansen).

It is thus apparent that these three species may not be closely differentiated by the shape of the cells. There is always a predominating form which attracts attention; with *S. cerevisiae* the predominating form is round; with *S. ellipsoideus* it is elliptical, while with *S. Pastorianus* it is most frequently elongated.

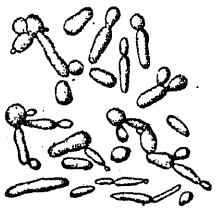


Fig. 3. — *Saccharomyces Pastorianus* (according to Hansen).

The majority of the yeasts, notably those of industrial importance (beer, alcohol, wine and cider), present a mixture of spherical and elongated cells. Although this is the case, a predominating form exists which may be of three types, the *cerevisiae* type, the *ellipsoideus* type or the *Pastorianus* type.

Among the yeasts, which are very numerous and in which the cell shapes are variable and indefinite, are often found certain species, or groups of species, in which the cells present a characteristic shape and which are separated closely from the preceding yeasts. *Hansenia apiculata*, for example, offers cells which are usually of the shape of a lemon, being provided with small projections from which the name *apiculata* is derived. A series of species of yeasts is known which possesses a similar shape and these, without doubt, are varieties of *Hansenia apiculata* or neighboring species. (See Fig. 4, *g*.)

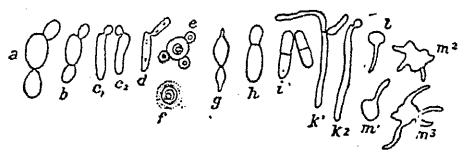


Fig. 4. — Showing the Different Shapes of Yeast Cells.

a, cerevisiae type; *b*, ellipsoideus; c^1, c^2, Pastorianus type; *d*, Mycoderma type; *e*, *f*, Torula type; *g*, apiculate type; *h*, Saccharomycodes type; *i*, Schizosaccharomyces; k^1, k^2, Mycelial structures; *l*, m^1, m^2, m^3, Amoeboid forms (according to Lindner).

Most of the *Torula* are easily recognized by their almost perfectly round shape, their content of fat, their peculiar manner of propagation which causes them to give off simultaneously many small round buds, and, finally, by their membrane, almost always surrounded by a layer of a mucilaginous substance. The genus *Torulaspora* and a few other yeasts have this same shape, which is known as the *Torula* type.

The *Mycoderma* and certain members of the genus *Pichia* often possess a decidedly refractive appearance, and elongated cylindrical cells which bud almost exclusively at two poles; their contents is transparent, enclosing a number of refractive granules localized especially in the extremities. This is the mycoderma type of yeast cell.

With *S. Ludwigii*, the cells possess a very peculiar form, tubuliform, bottle, or sausage shaped. Their division is intermediary between budding and partition.

Finally there are the *Schizosaccharomyces*, which are not ordinarily confused with other yeasts, for as the name indicates, they always multiply by division and not by budding. In *Sch. octosporus* the cells vary from a spherical form to the form of a drum stick. The spherical cells resemble huge micrococci while the drum-stick-shaped cells resemble the bacilli.

It is seen, then, that the yeasts present very common forms which are exhibited rather regularly by the various species. It is well to add that certain yeasts are able to assume abnormal forms. Thus, Lindner showed that *S. Bailii*, when growing in giant cultures on gelatin, resembled ameboid bodies.

Mycelial Formations

It has been stated before that the yeasts may grow in a filamentous or mycelial formation. Nevertheless it does not occur in all of the yeasts, and never appears where there is feeble development. It appears, however, only under special conditions.

Mycelial formation was observed for the first time by Hansen in the growth which covered the surface of fermenting liquids; this is termed a pellicle or scum. This growth presents a very different appearance from that which is found upon the bottom of flasks. Colonies are composed of long threads and cells and, little by little, the growth takes on a resemblance of a mycelium. The formations, however, always remain in a rudimentary state.

The investigations of Hansen, Lindner, and Will have shown that certain yeasts are equally capable of forming the mycelial-like structure when growing on gelatin. It manifests itself very well in *S. marxianus* and *carlsbergensis*, *Pichia membranaefaciens*, in *Zygo-*

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saccharomyces priorianus and *japonicus* and *S. Ludwigii*. In this last variety, Hansen has proved the production of a well-developed mycelium; however, this mycelium is rarely composed of elements which are solidly united. The cells are, however, separated by well-marked walls, and each is able to thrust out buds or to develop ascospores. Certain parts of the mycelium offer very abnormal forms.

With certain species, the growth which is formed at the bottom of a flask during fermentation has a tendency to produce filamentous formations. Thus with *S. marxianus* has been observed the formation of little flakes of mycelium which rest on the bottom of the flask or float lightly in the liquid. Recently Lepeschkin[1] secured with *Sch. Pombe* and *mellacei*, under certain conditions, the formation of little flocks presenting all of the characteristics of a mycelium.

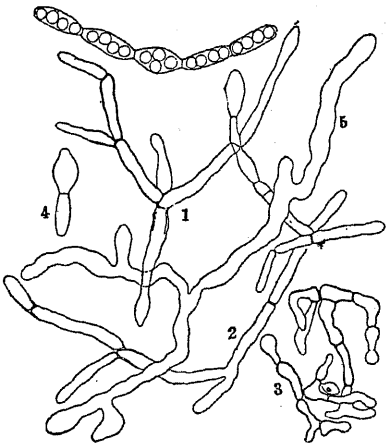


Fig. 5. — Mycelial Formations in Old Scums of *Saccharomyces Ludwigii* (according to Hansen).

Mycelial formations are found well developed in a yeast described by Guilliermond in 1917 under the name of yeast from Pulque No. 2.[2] A typical mycelium was formed of budding yeasts in most media, especially in the sediment which formed in beer wort as well as in the flocculent particles which float in the medium, on slices of carrot, and beer wort agar. The ascs seem to appear indifferently from the yeasts and from units in the mycelium.

CELL DIVISION

(A) Budding

Practically all of the yeasts divide by budding; it is the characteristic method for multiplication. The bud appears as a little prominence separated from the wall of the mother cell by a very narrow collar. Little by little it increases in size. When it has acquired a certain size, always smaller than the mother cell, it sepa-

[1] Lepeschkin, W. 1903. Zur Kenntnis der Erheblichkeit bei der einzelligen Organismen. Cent. Bakt. Abt. II, 10.

[2] Guilliermond, A. Levaduras del Pulque. Boletin de la Direccion de Estudios Biologicos. Mexico, ii, 1917.

rates. The daughter cell increases in size and soon equals that of the mother cell after which it, in turn, buds.

As has been said above, when multiplication is very active, each cell forms many buds simultaneously on different parts of its surface. It may happen that the buds attached to the cell which gave birth to them may begin to bud before an absolute breaking apart has taken place. This results in the formation of a small colony which is made up of a number of adhering cells. Depending upon the species, the cells appear united two by two, producing a colony made up of 15 or 20 cells. In general, top yeasts are distinguished from the bottom yeasts by the fact that the former remain united to one another, forming little chains, while the latter separate.

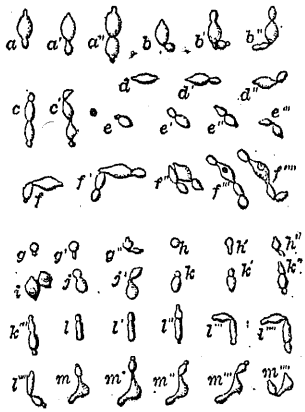


Fig. 6. — Budding in *Saccharomyces apiculatus* (according to Hansen).

With *S. apiculatus* and the genus *Hansenia* we have seen that the cells are provided at one or both of the extremities with little projections which give them the appearance of a lemon. It is interesting to observe how budding is accomplished in this yeast. Hansen has shown that the buds always form at the extremity of the cell.[1] The young bud may be apiculate at its free extremity, but it may be oval and give birth to oval buds deprived of points. This may be lost and the property of forming points again assumed.

(B) Transverse Division

The genus *Saccharomyces* presents a form of transition between the ordinary yeasts, which divide by budding, and the *Schizosaccharomyces*, in which division is accomplished transversely. In the *Saccharomyces* division consists of a sort of budding accompanied by the formation of a transverse partition, i.e., a process intermediary between budding and partition. The cells bud generally at their extremities; this is exceptional only when lateral budding is proven. Multiplication is often accomplished in the following manner: The cell elongates and at one end a sort of tube puffs out. This enlarges and is transformed slowly into a bud which remains united to the cell by a wide collar. A wall is formed across this which separates the cells from the bud.

[1] Hansen, E. C. Comp. Rend. des trav. du lab. de Carlsberg, 3, 1881.

True transverse partition is met only with the *Schizosaccharomyces* of which we shall mention the principal species.

Sch. octosporus possesses round or oval cells; in young cells the oval form predominates. They elongate and, after having acquired a certain size, form a wall across the middle. This splits apart and the two cells become rounded. They elongate when they have achieved their growth, and finally separate completely; but often the two cells, though remaining attached, undergo a new partition which makes two daughter cells. Thus a row of cells is secured which are arranged parallel to each other. Sometimes, when multiplication is very rapid, a primary transverse wall is formed which makes two cells; without separating, these produce another partition. In this manner, small filaments may be found which eventually break apart.

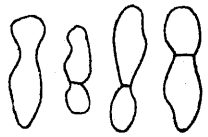


Fig. 7. — Budding in *Saccharomycodes Ludwigii.*

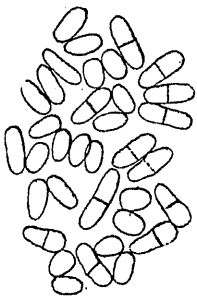


Fig. 8. — Showing Partition in *Schizosaccharomyces octosporus* (according to Schiönning).

At the end of some culture periods and also under certain conditions the cells show a tendency to take spherical forms. In this case partition is accomplished in the same manner; but it also often happens that, on account of rapid multiplication, the two cells set apart by a partition remain attached without rounding their adjacent planes. Each may then form transverse partitions which form two new cells. Such an arrangement resembles the sarcina grouping. In this latter case partition is accomplished in two directions.

The daughter cells remain associated for some time, giving the appearance of colonies. These colonies present different appearances, depending upon the age of the culture. In the early stages of their development these colonies are composed of elongated cells. Later muriform cells are apparent.

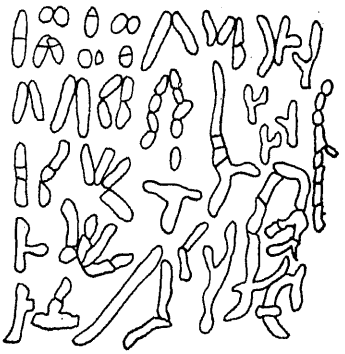


Fig. 9. — Partition in *Schizosaccharomyces Pombe* (according to Lindner).

In the other *Schizosaccharomyces* (*Sch. Pombe* and *Sch. mellacei*), in which the cells look like drum sticks, division is accomplished in the same manner. When they have acquired their maximum size, the cells form a partition which divides them into two cells. They either separate immediately or remain attached for some time.

In the latter case, they are able to turn about and, remaining attached, undergo a transverse division as with *Sch. octosporus*. They often remain completely adherent to form a chain with 2, 3, or 4 elements. Under certain conditions, notably in an environment with too little air, the cells show a very marked tendency of adhering together in chains which branch.

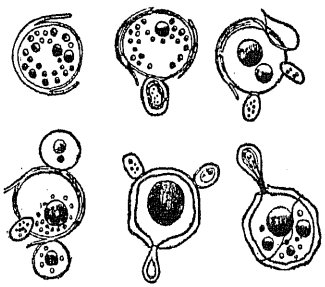


Fig. 10. — Durable Cells. The Membraneous Layer is Partly or Completely Detached (according to Will).

Sulc[1] has proven the existence in certain *Schizosaccharomyces* of certain fatty bodies. These *Schizosaccharomyces* multiply indifferently by partition or budding. This should be confirmed.

Durable Cells

In the pellicle which appears after a period of time on the surface of nutrient media, and in deposits at the bottom of flasks containing certain special media (sugar solutions containing tartaric acid or citric acid and mineral matter), Will[2] has observed cells which possess thick walls and whose contents are rich in glycogen and fats.

From the researches of Will and Casagrandi,[3] these cells possess a double membrane; the outer one is very fragile and easily broken to pieces. These membranes are made more visible by treating the cell with osmic acid or by the Ripart-Petit fluid (hydrochloric and chromic acids, 1 per cent).

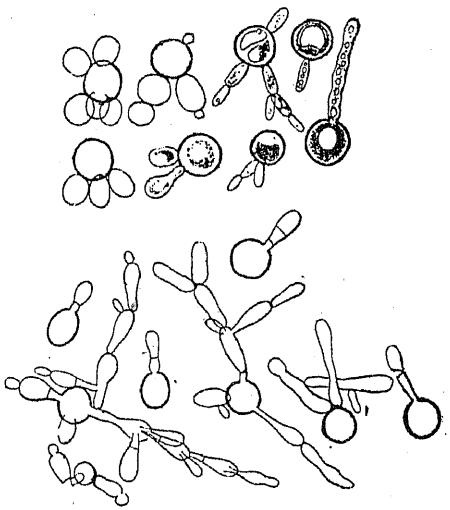


Fig. 11. — Germination of Durable Cells (according to Will).

Will has called these cells

[1] Sulc, K. "Pseudovitellius" und ähnliche Gewebe der Homopteren sind Wohnstätten symbiotischer Saccharomyceten (Sitzungsberichte der Königl. Böhm. Gesellschaft der Wissenschaften in Prag. March 30, 1910).

[2] Will, H. Vergleichende Untersuchungen an vier untergärigen Arten von Bierhefe. Zeitschr. f. d. ges. Brauwesen, Munich, II, 18, 1895.

[3] Casagrandi. 1897. Ueber Morphologie der Blastomyceten. Cent. Bakt., 3.

durable cells (Dauernzellen); he regards them as resistant organs which serve to perpetuate the species over unfavorable periods. In this, they are similar to the ascospores. Perhaps they may be regarded in the same light as cysts, or clamydospores, which have been observed so frequently in the *Endomyces*.

When these durable cells are placed again in favorable circumstances, their membrane is broken and budding takes place giving rise to spherical and elongated yeasts, be they separated or in groups.

Sporulation

Sporulation is a form of resistance which allows the yeast to remain viable, even though active budding has stopped. It plays an important rôle in the hibernation of yeasts, permitting them to pass the winter in the ground of vineyards where they are deposited in the autumn. Sporulation is observed in old cultures where food is scarce, also in certain solid media such as carrots or gelatin which are not very favorable for budding. It is especially easy to secure sporulation by submitting the yeasts to starvation after they have been able to build up sufficient reserve products necessary for the formation of ascospores.

We shall take up in a following chapter the details which determine sporulation; therefore this question will not receive attention at this time.

Internal, or ascospores, were observed for the first time by Schwann in 1839 and described by Seynes. They were regarded by some authors, notably Van Tieghm, as resulting from a sort of encystment resulting from some pathological process. Brefeld considered these cells, which bear spores, as sporangia or cysts. On the contrary, Rees,[1] de Bary,[2] and later Hansen,[3] likened the sporangia of yeasts to the asc of the *Ascomycetes* and regarded the yeasts as a group of fungi. This opinion has been entirely confirmed by our investigations on the cytological phenomena of the formation of ascospores, and especially by the discovery in certain yeasts of a copulation in the origin of the asc. It is definitely admitted today.

Sporulation is indicated by any cell, either yeast cell or a cell constituting a rudimentary mycelium. In this way certain yeasts (*S. Ludwigii, Pichia membranaefaciens*) are able to form ascospores in mycelial cells developing on the surface of old cultures. Each cell, then, seems able to develop into an asc.

[1] Rees, H. 1870. Botan. Untersuchungen über die Alcoholgärungspilze, Leipzig.

[2] De Bary, A. Morphologie des Pilzes. Leipzig, 1866.

[3] Hansen, E. C. Recherches sur la physiologie et la morphologie des ferments alcooliques. Comp. Rend. des trav. du lab. de Carlsberg, 2, 1883.

With the exception of the case, which we shall consider a little later, in which the asc results from a copulation, the ascs retain generally the form and dimensions of ordinary cells. (Fig. 12.) However, in *Nematospora coryli* and *Monospora cuspidata* the ascs are rectangular cells more elongated and larger than the vegetative cells.



Fig. 12. — Ascs of *Saccharomyces cerevisiae* (according to Hansen).

Often the ascs are derived from cells which have not ceased to bud. There are no clear-cut limits between budding and sporulation, for both are able to be carried on at the same time. Budding continues and slows up only at the time when sporulation begins. This explains why one often sees cells with ascospores and buds, the bud remaining attached to the mother cell and developing.

The number of ascospores contained in an asc is variable. It may vary between 1 and 12. However, it usually becomes fixed as in many of the industrial yeasts where a certain number usually predominates.

The number of ascospores in *S. cerevisiae* varies between 1 and 5, but 4 are more frequent. With *S. Pastorianus* the same variation obtains, but 2 ascospores are more common. With other yeasts, the number varies less widely and is more constant. With *Saccharomyces Ludwigii* and the yeast *Johanisberg II*, it is almost always 4. With *Sch. octosporus*, sometimes 4 and sometimes 8 ascospores may be counted. With *Sch. mellacei* and *Pombe* the number of ascospores is invariably 4. The same number obtains constantly with *Nematospora coryli*. *Monospora cuspidata* contains only a single spore in the asc. With *Debaryomyces globosus* and *Schwanniomyces occidentalis* the number varies between 1 and 2. Thus, with each yeast the asc tends to form a constant number of ascospores. This number varies, depending upon the species, from 1 to 2, 4, and rarely 8 or more.

The ascospores have dimensions between 1.5 and 5 μ. Usually they are spherical or oval (*S. cerevisiae, S. Pastorianus, S. ellipsoideus*). Sometimes they possess a globule of fat. The ascospores of some yeasts have characteristic forms. In *Willia anomala*, also in the genus *Hansenia*, the ascospores present a form quite similar to the cells of lower *Ascomycetes* (*Ascoidea rubescens, Endomyces decipiens*, and *Endomyces fibuliger*); they are hemispherical and their adjacent planes are provided with a projecting border which gives them the appearance of a hat. The ascospores of *Willia Saturnus* have the shape of a lemon and are girdled with a projecting ring. (Fig. 13, 3.) Cells of *Pichia membranaefaciens* have irregular shapes,

spherical, oval, elongated, triangular, kidney-shaped, or hemispherical. Sometimes they are small and hyaline, with a refractive globule in the center. In *Debaryomyces* and *Nadsonia* one finds globular ascospores enveloped in a membrane which is covered with stiff, erect protuberances. (Fig. 13, 5.) In *Schwanniomyces occidentalis*, the ascospores are provided with a projection about the cells which divides them into two unequal parts. (Fig. 13, 6.) The ascospores of *Schizosaccharomyces* may be ellipsoidal or elongated, and their membrane is impregnated with starchy materials which are stained blue with iodin-potassium iodide solution. *Nematospora coryli* possesses ascospores which are long and fusiform; they are provided with long

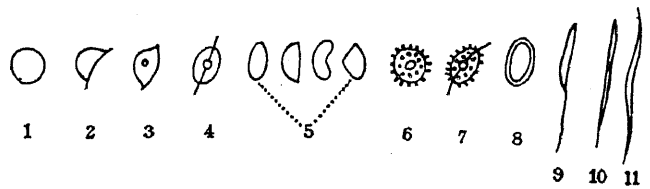


Fig. 13. — Showing the Various Shapes of Ascospores in Yeasts.

1, Ascospores from *S. cerevisiae;* 2, Ascospores from *Willia anomala;* 3, Ascospores from *Zygosaccharomyces chevalieri;* 4, Ascospores from *Willia Saturnus;* 5, Ascospores from *Pichia membranaefaciens;* 6, Ascospores from *Debaromyces;* 7, Ascospores from *Schwanniomyces occidentalis;* 8, Ascospores from *Saccharomyces capsularis;* 9, Ascospores from *Nematospora coryli;* 10, Ascospores from *Monospora cuspidata;* 11, Ascospores from *Coccidiascus Legeri.*

structures at their extremities which are similar to cilia. *Monospora cuspidata* also has long cells which look much like needles. (Fig. 13, 9.)

Most of the yeasts known possess only a single membrane. In *S. guttulatus*, there are, on the contrary, two membranes. The outer one breaks at the moment of germination.

SEXUALITY

(A) Copulation Preceding the Formation of the Asc

Recent investigations have shown that with a certain number of yeasts, the asc results from a copulation which closely resembles that of cells which one finds in certain of the lower *Ascomyces*. (*Eremascus* and *Endomyces*.)

It was among the *Schizosaccharomycetes* that this phenomenon was noticed for the first time. In this group of yeasts, characterized as we have seen by a special multiplication of cells which occurs always by transverse partition, only a few species are known (*Sch. octosporus, Sch. Pombe,* and *Sch. mellacei*). These three species show sexual processes.

In 1895, Schiönning[1] showed in a short note that the asci of *Sch. octosporus* resulted from the fusion of two sister cells, but not having observed the cytological phenomena which accompanied this fusion, he was not able to realize its significance. Hoffmeister[2] thought that he observed in this phenomenon a nuclear fusion, but at that time the nutrition of yeasts was insufficiently known to permit accurate observations. Bearing in mind the investigations of these two investigators, Guilliermond[3] has succeeded in demonstrating that this fusion is a true copulation. It is easy to observe this phenomenon in a Böttcher moist chamber in a drop of beer wort gelatin. The ascospores deposited in it are not slow to germinate and produce vegetative cells which multiply very actively during the first two days; toward the third day the multiplication decreases. The cells are then adherent in little colonies of 15 or 20, perhaps a few less. Some continue to divide, but most cease to multiply.

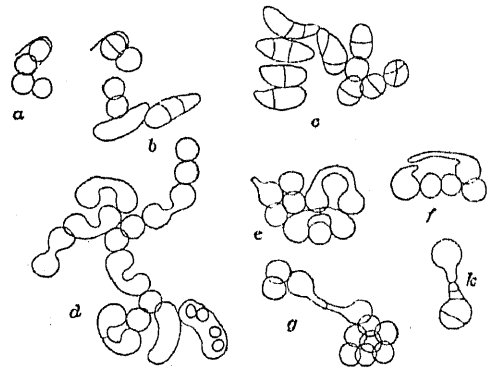


Fig. 14. — Different Stages in the Development of *Schizosaccharomyces octosporus*.

a, and *h*, germination of ascospores; *c*, multiplication of cells; *d*, copulation and formation of the asc; *e*, *f*, *g*, and *k*, copulation in a species tending to become asporogenic.

At this moment copulation commences and is accomplished in the following manner: Two cells identical in characteristics and lying adjacent to each other in the same colony are joined by means of a copulation canal, formed by the fusion of two little projections put out by each cell. (Fig. 15.) The middle wall which separates the two cells is rather quickly dissolved, and the nucleus of each cell, transformed thus into gametes, passes through the copulation canal. By this operation, a single cell, which is an egg or zygospore, is formed. Formed in this manner by isogamic copulation, the egg soon germinates. It increases in volume, while its nucleus undergoes two successive divisions, sometimes three, which give 4 or 8 nuclei. Then these become distributed about the zygospore and,

[1] Schiönning, H. 1895. Nouvelle et singulière formation d'ascus dans une levure. C. R. lab. de Carlsberg, 4.

[2] Hoffmeister, C. Zum Nachweiss des Zellkerns bei Saccharomyces. Sitzungsber. deutsch. naturw. mediz. Vereins f. Böhmen, 25, 1900.

[3] Guilliermond, A. Recherches histologiques sur la sporulation des Schizosaccharomycetes. Comp. Rend. Acad. Sci. 133, July 22, 1901. Recherches cytologiques sur les levures, Storck, ed. Lyon, 1902.

surrounding themselves with a zone of protoplasm, form 4 or 8 ascospores. The zygospore is then transformed into an asc. In many cases the fusion of gametes is not always complete and the asc retains a median constriction, a remnant of the copulation canal. It often happens that, in certain cases, the gametes remain individualized, and the asc may be constituted of two cells united by a copulation canal. In this case, the ascospores are formed in groups of 2 or 4 in each cell. These are formed especially when copulation takes place between cells which, not being united, are obliged to send out long projections. With *Sch. octosporus* all of the steps between complete and incomplete fusion of gametes may be observed, but in the two cases the result is the same and it produces a zygospore.

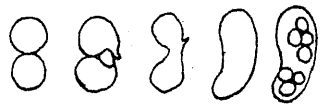


Fig. 15. — Various Stages in the Copulation of *Schizosaccharomyces octosporus* as Observed in Böttcher's Moist Chamber. *a*, 10 o'clock in the morning; *b*, 1 o'clock; *c*, 2 o'clock; *d*, 5 o'clock; *e*, 6 o'clock.

In a few rare cases the asc originates by parthenogenesis; this is happening when one sees two gametes united by a canal in which the wall has not been perforated, forming individually a parthogenetic asc.

A very peculiar fact, and one which should be mentioned here on account of its biological significance, is the copulation between two adjacent parent cells or, as Schiönning has described, between

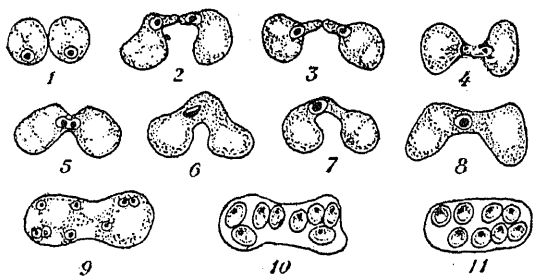


Fig. 16. — Copulation and Formation of the Asc in *Schizosaccharomyces octosporus* (in Stained Preparation).

two cells sprung from the same mother cell — between two brother gametes. It is a primitive characteristic which distinguishes this copulation from the sexuality of more highly developed organisms.

As a rule, almost all of the cells fuse two by two with the formation of the egg and more often between two adjacent cells in the same colony. As each colony is composed of 15 or 20 cells, the gametes are necessarily closely related. The same thing is true in colonies made up of 2 or 3 cells which are able to copulate two by two. One is able to follow under the microscope the formation of

a daughter cell which separates from the mother cell. This may unite with another daughter cell from the same parent to form an asc. However, as we shall see further on, the ascospores of the same asc, when in an environment unfavorable to multiplication, fuse two by two and are transformed into ascs without preliminary bipartition. In this case, as the asc always contains 4 or 8 ascospores, the gametes will not be separated by more than 3 or 4 generations.

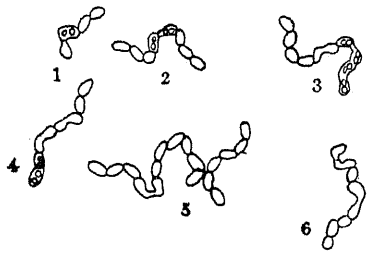


Fig. 17. — Different Stages in the Copulation and Formation of the Asc in *Schizosaccharomyces Pombe*.

Copulation may also be brought about between cells of very different parentage. As we shall see further on, Beijerinck has shown that *Sch. octosporus*, in continued cultivation in artificial media, may lose its properties of forming ascospores and becomes, after a long time, an asporogenous organism. In cultures undergoing this change, the number of asporogenous cells becomes greater and greater at the expense of the sporogenous cells. It happens that these latter cells are isolated in colonies in which all of the other cells have lost the sporogenic function. These, then, have to go to other colonies in their vicinity for sporogenous cells with which they are able to anastomose. They send out long tubes. These often go astray, form a wall across themselves and dissociate. From these facts, it is apparent that the parentage of the gametes is of little importance; as a rule gametes more or less closely situated fuse and copulation follows the law of least resistance.



Fig. 18. — Different Stages of Copulation in *Schizosaccharomyces Pombe* as Observed in Böttcher's Moist Chamber.

In *Sch. Pombe* and *Sch. mellacei*, two related species, copulation takes place in the same manner as in *Sch. octosporus*, with the only difference that fusion remains almost always incomplete. The cells destined to copulate are generally united in colonies of 4 to 20 cells situated in chains and presenting the form of small rods. Copulation is accomplished ordinarily between two cells adjacent in the same colony. (Figs. 17 and 18.) The gametes are united through a canal through which nuclear and protoplasmic fusion takes place. (Fig. 19.) The nucleus resulting from this fusion rather quickly divides, and the two nuclei thus formed emigrate to both enlargements of the zygospore where they undergo a second division necessary to the formation of ascospores. (Fig. 19.) The zygospore then is

transformed into an asc which preserves always the form of a dumb-bell. The ascospores, to the number of 4, originate in pairs in both enlargements.

Parthenogenesis, extremely rare in *Sch. octosporus*, is, on the contrary, rather frequent in *Sch. Pombe* and *Sch. mellacei*. Sometimes two cells, already united by a copulation canal, form, without reabsorbing the separating walls, a parthenogenetic asc; more often it is an ordinary cell, which, without trying to unite itself to another, transforms itself directly into an asc. (Fig. 17, 4.) Sulc has described a new species of *Schizosaccharomyces, Sch. Aphalarae calthae*, found in the fatty tissue of the homoptera, which seems to present a copulation analogous to that of *Sch. octosporus*. Nakazawa has found a copulation quite similar to that of *Sch. Pombe* and *mellacei* in *Sch. Sautanensis* and *formosensis* which were isolated by him from sugar products in Formosa.

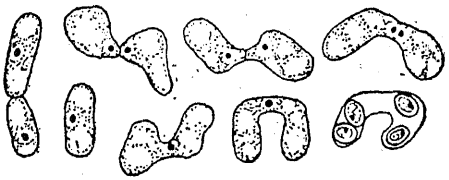


Fig. 19. — Various Stages in the Copulation and Formation of the Asc in *Schizosaccharomyces Pombe* (in Stained Preparation).

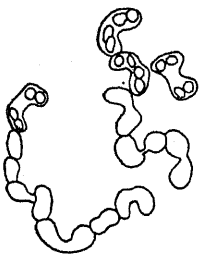


Fig. 20. — Copulation and Formation of the Asc in *Sch. mellacei*.

The sexual phenomena are present not only in the *Schizosaccharomyces;* they have been described also in a certain number of yeasts, multiplying by budding. One observes not only isogamy but heterogamy and intermediate forms between these two methods.

Barker,[1] in 1901, established the first of these in a new species isolated from a solution containing ginger, for which he created the genus *Zygosaccharomyces*. This yeast is known today as *Zyg. Barkeri*. The copulation is isogamic and occurs in the same manner as in *Sch. Pombe* and *mellacei*. The fusion is incomplete, and the asc which results retains the form of two retorts united by a collar. The mixture of the protoplasm and the nuclear fusion takes place in the copulation canal. The ascospores, which vary in number from two to four, develop in both enlargements of the asc or exceptionally in but one. (Fig. 21.)



Fig. 21. — Different Stages in the Copulation and Formation of the Asc in *Zygosaccharomyces Barkeri* (afterBarker).

Recent work has shown that these sexual phenomena, which have been regarded as rare at the time when they were first observed, are

[1] Barker, P. A conjugating yeast. Proc. of the Roy. Soc. 68, July 8, 1901. On the spore formation among the saccharomycetes. Jour. Federated Institutes of Brewing, 13, 1902.

really quite widespread among the yeasts. Since the discovery of *Zyg. Barkeri*, Klöcker [1] has recorded the existence of a similar copulation in *Zyg. Priorianus* and *Zyg. mandshuricus*, de Kruyff [2] in *Zyg. javanicus*, Saito [3] in *Zyg. japonicus*, Dombrowski [4] in *Zyg. lactis*, Takahashi in *Zyg. Major*, Richter in *Zyg. mellis acidi*, and Pearse and Barker [5] in a yeast from cider provisionally designated as "Yeast F." Chatton has also described an isogamic copulation in a yeast isolated from *Drosophilia funebris* which he names *Coccidiascus Legeri*.[6] It seems that this copulation existed among other yeasts but had not received the attention of authors who described them.

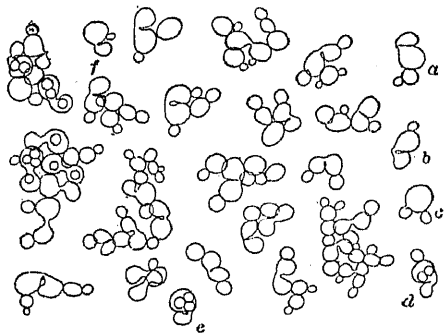


Fig. 22. — Copulation and Formation of the Asc in *Zygosaccharomyces Priorianus*.

Among all of these yeasts, copulation occurs exactly as in *Sch. Pombe* and *mellacei*. It obtains between adjacent cells and very closely related parents. With *Zyg. Priorianus*, for example, copulation almost always takes place between two cells of the same colony made up of 15 or 20 cells. Sometimes it takes place between cells in a colony composed of only 3 or 4 cells. It happens also that one is able to see a cell in the act of forming a bud and also fusing with the latter before it has achieved its full development. In this case, the asc which results is composed of two unequal enlargements; one, the larger, representing the mother cell, and the other representing the bud. The ascospores not having sufficient space for germination into the bud, form themselves uniquely in the mother cell. In this manner, copulation normally isogamic finds itself heterogamic. Perhaps we may see in this anomaly a tendency to heterogamy.

[1] Klöcker, A., in Lafar Handbuch der Technischen Mykologie. G. Fischer, Jena, 1905.

[2] de Kruyff, E. Untersuchungen über auf Java einheimische Hefenarten, Cent. Bakt. 21, 1908.

[3] Saito, K. Preliminary notes on the spore formation on the so-called "Soya Kahmhefe." Botanical Mag. 13, 1909.

[4] Dombrowski, W. Die Hefen in Milch und Milchprodukten. Cent. Bakt. Abt. II, 28, 1910.

[5] Pearse, B., and Barker, P. The yeast flora of bottled ciders. Jour. Ag. Sci. 3, 1908.

[6] Guilliermond, A. Quelques remarques sur la copulation des levures. Annales mycologici, 8, 1910.

Another yeast isolated by Pearse and Barker[1] from cider and designated Yeast G presents a copulation which is intermediary between isogamy and heterogamy. In this species the two gametes are cells of the same dimensions which do not show morphologically any sexual differentiation. But the contents of one, which may be regarded as the male, pass into the other, which may be regarded as the female. The ascospores originate from this last and they are always to the number of two. (Fig. 23.)

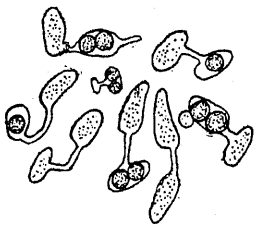


Fig. 23. — Copulation in "Yeast G" of Pearse and Barker (according to Pearse and Barker).

More recently, a strictly heterogamic process was observed in a new species isolated from fermentation products of wine from Bili.[2] This yeast, which we have named *Zygosaccharomyces chevalieri*, has ascs which result from a copulation between two cells of different dimensions. (Fig. 24.) One is very small and represents the male gamete. It is young, while the other, representing the female gamete, is larger and much older, having attained its full development. The two cells unite by means of a copulation canal and the contents of the male gamete pass into the female gamete in which the protoplasmic and nuclear fusion takes place. After this has taken place, the female gamete separates itself by means of a wall, and produces from 1 to 4 ascospores. During this the membrane of the male gamete is absorbed. It is also rare to observe an adult asc again united to a male gamete.

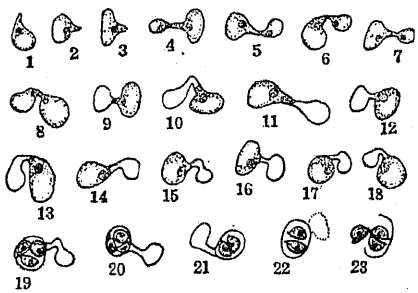


Fig. 24. — Various Stages in the Copulation and Formation of the Asc in Yeast Bili 2.

1–3, gametes sending out tubules destined for copulation; 4–8, union of two gametes by a copulation canal; 9–18, the contents of the male gamete mixes with the contents of the female gamete which changes into an egg; 19–21, formation of ascospores; 22, adult asc; 23, asc breaking its membrane and freeing ascospores.

Recent investigations have shown that the heterogamic copulation occurs frequently. In *Zygosaccharomyces priorianus* it has been shown that copulation is accomplished sometimes, but rarely, between an adult cell and a bud formed by it. In *Debaromyces globosus* Guilliermond has shown that there takes place a heterogamic copula-

[1] Pearse, B., and Barker, P. The yeast flora of bottled ciders. Jour. Agricultural Science, 3, 1908.

[2] Guilliermond, A. Sur un exemple de copulation hétérogamique observé dans une levure. Comp. Rend. de la Soc. Biologie, 70, 1911.

tion between a mother cell and a bud formed by that cell, which is very small and still attached to the mother cell. About 25 per cent, only, of the asces result from an isogamic copulation between two cells of the same dimensions. In *Debaromyces tyrocolla*, the copulation is accomplished most often between the mother cell and its bud and the isogamic copulation is very rare. According to Konokotin, the genus *Debaromyces* may be represented by a heterogamic copulation. In one other yeast, isolated by Guilliermond from oranges, the *Zygosaccharomyces Nadsonii*, a heterogamic copulation has been described, but with this yeast the heterogamy is the rule, and there seems to be no instances of isogamy.

Finally Nadson and Konokotin have discovered in the mucous secretions of trees two species of yeasts (*Nadsonia fulvescens* and *elongata*) in which the copulation always takes place by heterogamy between an adult cell and one of the buds formed by it. All of the contents of the male gamete go into the female gamete, but the female gamete does not transform directly into an asc. It gives birth, by budding, to a new cell into which its contents are poured, and it is this cell which becomes the asc, usually including a single ascospore. The authors think that the asc, formed by budding, represents a rudiment of a sporophyte, and consequently have established for these two species the genus *Nadsonia* (*Guilliermondia*). With *Zygosaccharomyces* it has been difficult to determine the parents of the gametes which unite; the copulation is almost always accomplished between an adult cell and a young cell. In other yeasts with heterogamic copulation, it takes place between a mother cell and one of the cells formed by budding from it. In this case copulation may be autogamic.

Guilliermond[1] has isolated from the mucous secretions of chestnut trees a new yeast, *Zygosaccharomyces Pastori*,[2] which presents a heterogamic copulation effected between cells of unequal sizes. The female cell is the adult while the other, the male, has not attained its full development. Sometimes there is little difference between the two cells. The contents of one always pour into the other which is transformed into the asc. The asc may contain from 1 to 4 ascospores which are hat-shaped like those of *Willia anomala*. This yeast produces only a few asces; the cells, however, attempt to unite

[1] Guilliermond, A. Sur une nouvelle levure à copulation hétérogamique. Comp. Rend. Soc. Biol. 1919.

[2] This yeast has not been fully described but presents a resemblance to *Willia anomala* in having hat-shaped ascospores. Its cultural characteristics, however, do not class it in the group *Willia*. Klöcker has described an apiculate yeast in the *Saccharomyces apiculatus* group in which the spores are hat-shaped. This form ought not to be regarded as a characteristic of the group *Willia*

with others by means of small projecting tubes, some cells possessing many of them, but this is hardly ever successful. It is then possible that we are concerned with yeast which is in the process of losing its sexuality and sporogenic function.

Cesari[1] has recently isolated a series of yeasts from sausage and salted meats which seems to act on albuminous matter and play a rôle in the ripening process. All of these yeasts possess a heterogamic copulation resulting in the formation of an asc with a single ascospore.

(B) Copulation of Ascospores or Parthenogamy

The copulation which has just been described, is not the only form of sexuality observed among the yeasts. In certain species occurs a sexually different process which is brought about by a subsequent stage in the germination of ascospores. It is thus that in *S. Ludwigii* and *Willia Saturnus* and the yeast Johannisberg II, an isogamic copulation between ascospores originating from an asc formed without fusion, has been established. This phenomenon will be discussed at this time without entering into a detailed discussion of the germination of ascospores.

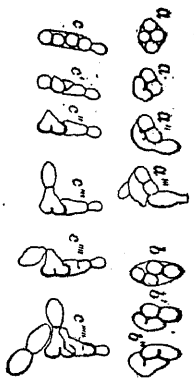


Fig. 25. — Various Stages in the Germination of Ascospores in *S. Ludwigii* as Observed in a Moist Chamber (according to Hansen).

In *S. Ludwigii* the asc contains almost always four ascospores; at the moment of copulation, these ascospores copulate two by two by means of a copulation canal formed by the fusion of two little projections from each cell. (Fig. 25.) It was Hansen[2] who showed for the first time the existence of this phenomenon. It is shown later that this fusion presents the characteristics of true copulation and that it is accompanied by a nuclear fusion.

The nucleus and cytoplasm are introduced into the copulation canal and it is there that the mixture of nucleus and cytoplasm takes place. The fusion remains incomplete and the zygospore is formed from two ascospores united by a copulation canal. In this canal, the egg is formed which brings about the germination of the zygospore. It elongates into a germination tube from which originate numerous vegetative cells.

Copulation is accomplished normally between two ascospores of the same asc before the partition is absorbed. Buds result from the

[1] Cesari, E.-P. La maturation du saucisson. Comp. Rend. Soc. Biol., 1919.

[2] Hansen, E. C. Recherches sur la morphologie et la physiologie des ferments alcooliques, 3, 1891.

germination of the zygospore which, in developing, perforate the wall of the asc. The copulation of the asc is always by autogamy and, as each asc contains only four ascospores, it will be able to occur only between sister ascospores. However, by force of circumstances copulation may also be accomplished between ascospores from different ascs and consequently from more distant relationships. This is almost constantly met with when one makes old ascospores germinate; under these circumstances, a great number between them are dead and those which have survived are among others which are not capable of development. On account of this they will be obliged to search in other ascs for ascospores with which to unite. They accomplish their union by means of long organs.

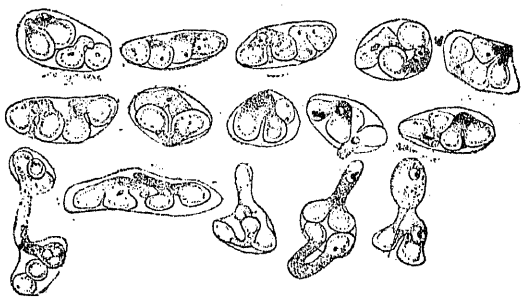


Fig. 26. — Various Stages in the Copulation of the Ascospores in *Saccharomyces Ludwigii*.

This copulation is accompanied by numerous parthenogeneses. About one-fourth of the ascospores germinate without undergoing copulation. The analogous phenomenon has been found in *Willia Saturnus* and in the yeast Johannisberg II (Fig. 27), but for these two species parthenogenesis is still most frequent, and half of the ascospores germinate without copulation.

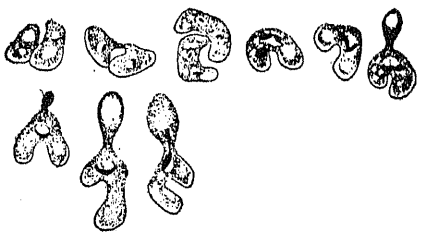


Fig. 27. — Various Stages in the Copulation of Ascospores in Yeast Johannisberg II.

This second form of copulation seems to be quite common among the yeasts. H. Marchand has found this copulation in many yeasts (*S. intermedius, turbidans, validus, vini Muntzii, Johannisberg I, S. Willianus*). In these yeasts about one-half of the ascospores germinate after having fused two by two; in *Saccharomyces validus*, however, this copulation is accomplished more rarely and becomes exceptional. Guilliermond has observed the copulation of ascospores in three yeasts reported on and secured from the Chevalier mission (*Saccharomyces Mangani, Lindnerii* and *Chevalieri*), and also the yeast from Pulque No. 2. It has been found by Kinokotin in *Saccharomyces paradoxus*, but in this yeast it presents very special characteristics, the interpretation of which is rather close. Lindner

has noticed this in another yeast which he has not named. This was isolated from the mucilaginous secretions on a tree in the Berlin Botanical Garden.

Copulation of ascospores does not seem to be regarded as a true fecundation but as a phenomenon[1] of parthenogamy — a sexual process replacing fecundation. In fact, it may be admitted that the copulation which takes place in *Schizosaccharomyces*, and *Zygosaccharomyces* and *Debaromyces* at the moment when the asc forms represents a normal sexual process of yeasts. The copulation which takes place, then, among the ascospores, is a new process and one which takes the place of normal fecundation.

The cell which gives rise to the asc ought to be regarded as a gamete developing parthenogenetically. As the formation of ascospores necessitates two successive divisions which are not separated by a period of intercalary nutrition, the nucleus which results is quite devitalized. This may explain why the ascospores felt the need of compensating for the loss of chromatin which the nucleus has experienced in successive divisions. It is probable, however, from what is known with regard to the higher ascomycetes, that the asc of the yeasts is the seat of a reduction in the number of chromosomes. The copulation of ascospores may intervene to replace the fecundation which should occur at the moment when the ascs are formed and to compensate the loss of chromatin undergone in the course of mitosis of the asc.

(C) Retrogradation of Copulation — Parthenogenesis

As has been pointed out, in the great majority of yeasts, especially those which are of industrial significance, one does not find any trace of sexuality. As among species which present a copulation at the moment when the asc is formed, it has become established from numerous cases of parthenogenesis that the yeasts which do not have sexuality, represent parthenogenetic forms derived from primitive sex forms. The asc, when it has not resulted from a copulation, has then the import of a gamete having developed by parthenogenesis, that is, a parthenospore. The yeasts may be regarded as a group of fungi which have gone toward parthenogenesis by evolution or by force of unknown circumstances, and which are

[1] This phenomenon is comparable to that which Brauer has observed in the parthenogenesis of an echinoderm, *Artemia salina*. In this organism, when fecundation has not taken place, there is a fusion of a second polar globule with the egg, and this fusion takes the place of fecundation. Some phenomena which appear to be similar have been found since among various fungi and protozoa and have been grouped under the name of parthenogamy.

losing their sexuality. This opinion is supported by a series of very striking facts which are quite apparent. A consideration of the development of various members of this group will give the proof of progressive disappearance which sexuality in yeasts has undergone.

In the *Schizosaccharomyces*, which present in this connection very interesting characteristics, copulation is illustrated in three varieties: *Sch. octosporus*, *Sch. Pombe*, and *Sch. mellacei*. In the first copulation is almost universal. With the other two, on the contrary, copulation is rather frequent and a great number of cells sporulate without copulation. A species of *Schizosaccharomyces* has been transmitted from the laboratory of Professor Beijerinck under the name of *Sch. mellacei*, which did not present any trace of sexuality; the ascospores originated in ordinary cells which had not undergone copulation. This yeast, which resembled *Sch. mellacei* very closely, may have been another variety.



Fig. 28. — Parthenogenetic Ascs in *Debaromyces globosus*.

Among the different species of the genus *Zygosaccharomyces* numerous cases of parthenogenesis have been observed. With *Debaromyces globosus*, however, this characteristic is more predominant and many ascs originate without copulation. These may be formed by ordinary cells or cells with long projections giving them the shape of dumb-bells. (Fig. 28.)

Some yeasts have lost their sexual characteristics but have retained traces. Such is the case with *Schwanniomyces occidentalis* which has been described by Klöcker. Guilliermond has shown that at the moment of sporulation in this yeast, the cells destined to form the ascs emit projections of different length by means of which they try to unite two by two. But the sexual attraction seems to disappear; it is only exceptional that they join. These little projections, then, may be the remnants of an ancestral sexuality.

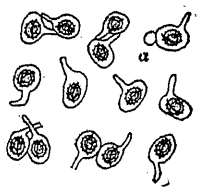


Fig. 29. — Formation of Asc in *Schwanniomyces occidentalis*.

Since then, Ludwig Rose[1] and Dombrowski[2] have observed the same characteristics, one in *Torulaspora Delbrücki*, in a new species related to this latter and in a new yeast isolated from the mucilaginous secretions from an oak tree, which was provisionally named Yeast F; the other in a milk yeast, *S. lactis*. Yeast F was studied by

[1] Ludwig Rose. Beiträge zur Kenntniss der Organismen in Eichenschleimfluss. Inaugural Dissertation, University Berlin, 1910.

[2] Dombrowski, W. Die Hefen in Milch und Milchprodukten. Cent. Bakt. Abt. II, 28, 1910

Guilliermond and was found to present a series of curious characteristics.[1] It forms only a few ascospores and it looks as if its sporogenic function is on the verge of extinction. However, when the yeast is placed in media suitable for spore formation, almost all of the cells put out long projections by means of which they attempt to anastomose two by two. Often these do not join together, as if there were an opposing force at work, or as if by a loss of sexual attraction, these projections when they come in contact continue to elongate and thus form a network. (Fig. 30.) In quite a number of cases some may establish a union for anastomosis by means of their projections and adhere sufficiently so that a slight pressure does not cause them to break apart. The wall which separates the two cells never quite disappears and in each case fusion does not take place. (Fig. 30, *a*.)

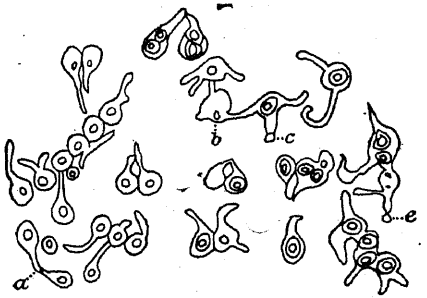


Fig. 30. — Formation of the Asc in "Yeast F" of Rose.

Occasionally, the projections from the cells undergo an excessive elongation, since, not having accomplished their function, they form a little bud at their extremity. Often a cell may give forth many little projections at different points on its surface in different directions and even these are capable of ramification. In this manner very peculiarly shaped cells are secured which look like amebae. Without much doubt Lindner observed analogous forms in the pellicle of cultures of *Saccharomyces Bailii*. (Fig. 31.) The forms of copulation, depicted by this author, seem to demonstrate the existence of a copulation in this yeast. The ameboid cells represent, then, unsuccessful copulation.

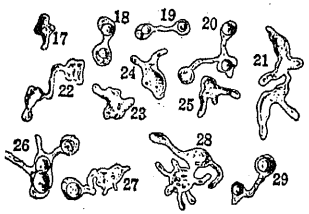


Fig. 31. — Ameboid Forms of *S. Bailii* in an Old Culture on Nutrient Gelatin. Some of the Forms have Sporulated (after Lindner).

Only a certain number of the cells which have just been described, about 28 per cent, enter the asc stage. All of the others become degenerate forms. The ascospores, in the number of from 1 to 4, originate in the body of the cell; but they are able to enter the interior of the projection which assumes a bulged form.

[1] Guilliermond, A. Sur la régression de la sexualité dans les levures. Soc. de Biol. 70, 1911. Sur la reproduction de Debaromyces globosus et sur quelques phénomènes de rétrogradation de la sexualité des levures. Comp. Rend. Acad. des Sciences, 151, 1911.

Thus, these examples indicate that, in many of the yeasts, the ascogenous cells which represent gametes, develop by parthenogenesis, preserving, nevertheless, a little of their sexual attraction; this is insufficiently developed to insure copulation. These species include the yeasts which have completely lost all traces of sexuality, and in which sexuality is definitely established, such as the *Saccharomyces* and the majority of yeasts.

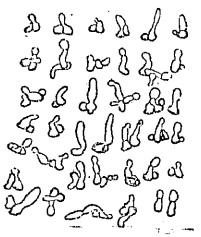


Fig. 32. — Parthenogenetic Variety of *Saccharomyces Ludwigii*. Germination of Ascospores.

We have seen that certain yeasts, as *S. Ludwigii*, *Johannisberg II* and *Willia Saturnus*, after having lost their primitive sexuality, have experienced the need of compensating this by a parthenogamy which consists in the nuclear and protoplasmic fusion of ascospores, two by two. But even this secondary sexuality seems to disappear. Thus in *Saccharomyces Ludwigii* about one-fourth of the spores germinate without copulation. With regard to the yeast Johannisberg II, and *Willia Saturnus*, parthenogamy is observed in only one-half of the ascospores. We have had opportunity to study a variety of yeast *Saccharomyces Ludwigii*, arising from a culture of Hansen's, which did not offer any trace of parthenogamy. The ascospores formed long projections which attempted to join but never accomplished this end. (Fig. 32.)

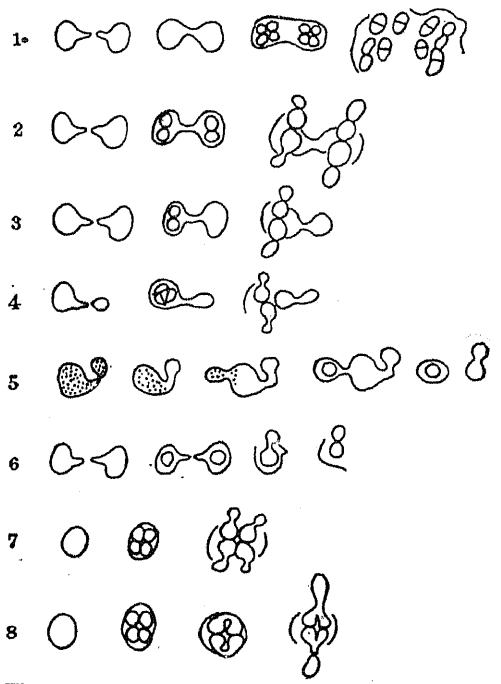


Fig. 33. — Scheme Representing the Development of Forms of Yeasts.

1, *Sch. octosporus* (isogamic); 2, *Zygosaccharomyces Barkeri* (isogamic); 3, "Yeast 6" of Pearse and Barker (intermediate forms between iso- and heterogamy); 4, *Zygosacch. chevalieri* (heterogamy); 5, *Nadsonia* (heterogamy and ascs resulting from the budding of eggs); 6, Yeast of Rose (parthenogamy with traces of sexual attraction); 7, *S. cerevisiae*, (parthenogenesis); 8, *S. Ludwigii* (parthenogamy between spores).

All this shows in an exact manner that the yeasts make one of very many examples of a group in which sexuality is in the act of retrograding and in which one may follow each step in the accomplishment of this phenomenon. From this point of

view, the yeasts are comparable to *Saprolegniaceae*, in which Barker has pointed out a similar process.

If one glances over the Saccharomyces, he will be able to distinguish four steps in the progressive evolution of sexuality (Fig. 33): firstly, those which have preserved ancestral copulation in origin of the asc (*Schizosaccharomyces, Zygosaccharomyces* and *Debaromyces globosus*); secondly, those which have lost this characteristic but may have kept traces of it (*Schwanniomyces occidentalis, Torulaspora Delbrücki*, and the yeast of L. Rose); thirdly, those which have lost ancestral copulation and replaced it by a parthenogamy between ascospores (Johannisberg II, *Willia Saturnus* and *S. Ludwigii*); fourthly, those which have lost all traces of copulation and have become parthenogenetic.

Germination of Ascospores

When placed under favorable conditions, ascospores germinate and produce numerous vegetative cells. The manner of this is different and depends upon the species.

First Example, *Saccharomyces cerevisiae* (Fig. 34): Let us start this discussion with *S. cerevisiae*, which has been studied by Hansen.[1] In the first phases of germination, the ascospores undergo a swelling but the wall subsists. This swelling is so great that the ascospores, by means of the pressure which they exert on one another, give the impression that the asc is chambered. In fact, the walls of the ascospores enter into intimate contact, and often they fuse completely in such a way that there are really walls in the asc which then becomes a cell with many chambers. (Fig. 34, *c*, *d*, *f*, and *g*.) During this time the wall of the asc becomes thinner and finally breaks. It acts as a plaited veil which retains the ascospores, or is completely absorbed by the ascospores. Each ascospore then takes the form of

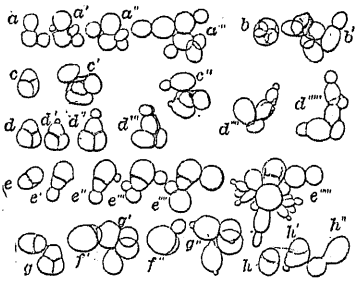


Fig. 34. — Germination of Ascospores in *Saccharomyces cerevisiae* (Observations from a Böttcher Moist Chamber).

a, three ascospores in an asc, the wall of which has broken; *a'* and *a''*, germination of these three ascospores; *b*, asc with four ascospores; *b'*, germination of these four ascospores; the wall of the asc is broken; *c*, asc with four ascospores, three of which are visible; *c'* and *c''*, germination of these ascospores; in *c'* the wall of the asc is broken, in *c''* budding has commenced; *d*, asc with three ascospores; *d* and *d'*, germination of these ascospores; in *d''* the wall of the asc is broken; *e*, *e'*, *e''*, *e'''*, *e''''*, various stages in the germination of two ascospores in an asc; in *e'''''* the two ascospores have fused into a single one by absorbing the separating wall; *f*, *f'*, *f''*, *g*, *g'*, *g''*, germination of ascospores in two ascs; *h*, *h'*, and *h''*, germination of two ascospores in an asc; in *h''* the wall between the two ascospores has disappeared; a single cell is thus formed (after Hansen).

[1] Hansen, E. C. Recherches sur la morphologie et physiologie des ferments alcooliques, 3, 1891.

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an ordinary vegetative cell. It commences to form a bud at some point on its surface (*d*, *e*, and *f*). This bud generally appears after the rupture or the absorption of the wall of the asc, but it may appear on the interior of the asc. Its appearance is soon followed by the formation of new buds which are formed at various parts of the surface of the ascospore. During the formation of these buds, the ascospores remain united but separate rapidly. Finally these germinate; the ascospores swell up and bud quickly after the manner of a vegetative cell.

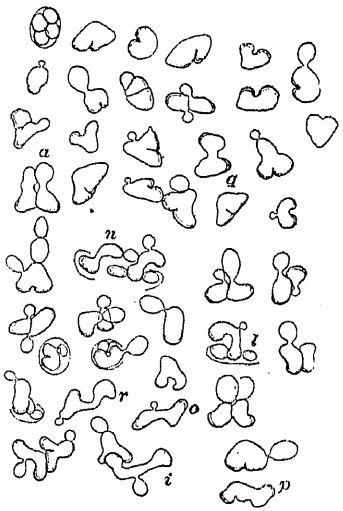


Fig. 35. — Germination of Ascospores in Yeast Johannisberg II.

a, two ascospores germinate without fusing; *e*, *n*, *l* and *p*, copulation between ascospores not included in the same asc.

Hansen has often observed, during budding, the fusion of two ascospores in a cell. But this fusion, which only appears in an exceptional manner, is not comparable to copulation which has been described in certain yeasts, as *S. Ludwigii*. It takes place only after the ascospores have commenced to bud, generally between an ascospore which has already formed many buds, and an ascospore which is not yet developed. Hansen supposed that one served to nourish the other, and that a case of parasitism was involved.

In the majority of yeasts, notably in *Saccharomyces Pastorianus* and in many of the industrial yeasts, germination occurs in the same manner as in *S. cerevisiae*. However in certain species, germination of ascospores is preceded by a copulation (parthenogamy); this is the case with yeasts already mentioned, as Johannisberg II, *S. intermedius*, *turbidans*, and *ellipsoideus*. It will be recalled that in this species the ascospores, after swelling up, unite two by two by means of a copulation canal. A zygospore is thus formed by the fusion of two ascospores. Budding takes place at the expense of the copulation canal. (Fig. 35.) It is produced at some point on its surface. Often many buds appear simultaneously at different points on the canal of copulation. Eventually, it happens that the buds originate at the expense of the ascospores themselves. In the mean-

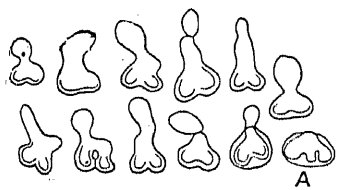


Fig. 36. — Germination of Ascospores in *Saccharomyces Ludwigii*.

In *A*, Fusion of Three Ascospores.

time, about one-half of the ascospores undergo this copulation; the others germinate by themselves without fusion.

Second Example, *Saccharomycodes*: The ascospores of the genus *Saccharomycodes*, which has been described for the first time by Hansen in *Saccharomyces Ludwigii*, germinate in a somewhat specialized manner. As we have seen, the ascospores of this yeast are almost constantly in the number of four in each asc. The wall of the asc is able to break before the germination to free the ascospores. More often they persist during the first phases. Germination begins always by a swelling of the ascospores. Whether these spring from old or young cultures, has much to do with the development.

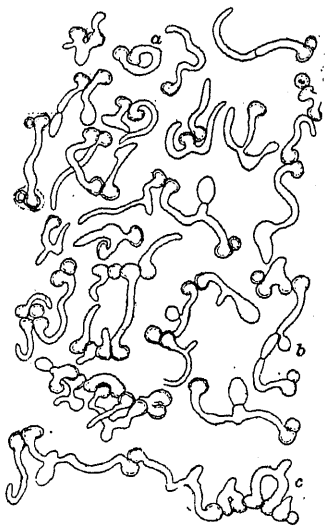


Fig. 37. — Germination of very Old Ascospores in *Saccharomyces Ludwigii*.

In the first case, the majority of ascospores, a little swollen, undergo a copulation which has been described in a preceding paragraph and upon which we shall not dwell at this time. The ascospores, ordinarily united in ascs in which the membrane is not broken, put out a little protuberance by means of which they unite two by two. The middle wall by which they are separated rather quickly disappears. Sometimes copulation takes place slowly; the protuberances put out by each cell elongate and fuse at the ends after having gone along together for some time. The ascospore then looks like a horse-shoe. (Fig. 37, *a* and *c*.) In some cases, one sees the fusion of three ascospores in the same asc in a single zygospore (Fig. 36, *A*). This, however, is very rare.

The copulation of the ascospores being incomplete, the zygospore is made up of two enlargements united by a copulation canal in which is concentrated the nucleus and protoplasm. This commences to germinate by a procedure intermediate between budding and transverse division. The center of this canal elongates into a little germination tube. This tube perforates the wall of the asc if it is not already absorbed, until it swells in its upper part. This then separates itself from the rest of the germination tube by a little wall and a slight circular constriction. The cell formed in this manner detaches itself from the zygospore, which forms new cells by the same procedure. Sometimes, the first cell formed by the zygospore, without detaching itself, gives birth to one or many more cells which

remain attached to one another, making a chain. With rare exceptions, the copulation canal of the zygospore germinates always in the same way. The simultaneous production of many buds is not observed at many points on the surface as with the yeast Johannisberg II.

About four ascospores germinate alone without preliminary copulation. In this case, after swelling, they form a new germinating tube in which the end is enlarged and take the form of an ordinary vegetative cell. Here again germination takes place only in a single direction and the ascospore forms only a single germinating tube at a time.

The germination of old ascospores is accomplished in a different manner. Hansen has observed that old ascospores, whether due to humidity or dryness, lose their tendency to fuse and germinate alone. They develop, then, in a peculiar manner. Each forms a germinating tube which, in developing, produces a long filamentous form of very many cells superimposed and capable of ramifying. It presents something the appearance of a mycelium. Hansen has given the name *promycelium* to this formation and compared it to the filaments which result from the germination of chlamydospores of the *Ustilaginales*. Guilliermond has verified this observation in the germination of ascospores from old cultures and his observations have allowed an explanation of this structure, improperly called a promycelium. As has been said in the preceding paragraph, the ascospores from very old cultures find themselves obstructed in copulation. A great number are dead, and the cells which survive are often isolated and surrounded by spores which are incapable of developing. On account of this they may have to search other ascs with which to unite. They send out long tubes more or less branched which may accomplish a fusion but which more often do not unite. In this case the tubes end up by walling off cells which dissociate and take the form of vegetative cells. Under such conditions the greater part of the ascospores find it necessary to germinate alone. One usually finds a few which are able to copulate.

Let us recall what we have described in *S. Ludwigii*, in which the ascospores always germinate without preliminary copulation. However, many of them preserve their traces of sexual attraction, and send out, in germinating, long protuberances which do not accomplish anything.

The germination of the ascospores of *S. Ludwigii* differs essentially from the other yeasts, and in this one the ascospores, copulated or not, do not produce many buds at various points on the surface, but germinate in a single direction in which they form a sort of

germination tube. This separates the ascospore by a transverse wall accompanied by a slight constriction. In this the germination of the ascospores does not differ from the method of multiplication of cells in this yeast which, as we have seen, generally occurs at the end of the cell by a process intermediary between budding and transverse partition.

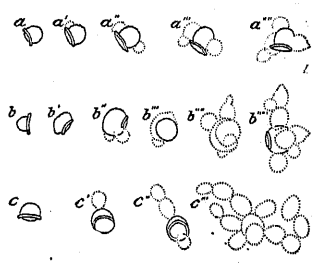


Fig. 38. — Germination of Ascospores in *Willia anomala* (after Hansen).

Third Example, *Willia:* We have seen that the ascospores of the genus *Willia* present a special form. In *Willia anomala* they are hemispherical and are provided with a projecting edge. (Fig. 38, *a.*) At the moment of germination, which has been followed by Hansen, the ascospore swells and during this its jutting out border disappears but remains for some time during the early stages of budding. The ascospore eventually forms buds at different points on its surface.

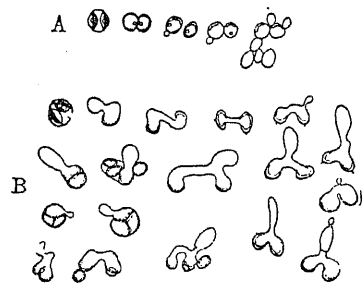


Fig. 39. — Germination of Ascospores in *Willia Saturnus.*

In *Willia Saturnus* the ascospores are lemon-shaped and are girdled with a projecting ring. The wall of the asc generally dissolves before germination. This begins by a swelling during which the projecting girdle disappears or remains; then a series of buds is produced at various points on the surface of the ascospore. In the course of budding, the projecting thread disappears. (Klöcker.[1]) A. In many cases germination is preceded, as has been stated in a foregoing paragraph, by a parthenogamic copulation of the ascospores. These, during enlargement, unite two by two by means of a copulation canal. B. The fusion is incomplete and the zygospore which results germinates by budding on all points of its surface, by preference on the copulation canal.

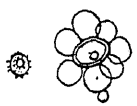


Fig. 40.— Germination of Ascospores in *Debaromyces globosus* (after Klöcker).

Fourth Example, *Debaryomyces globosus* and *Schwanniomyces occidentalis.* Both of these yeasts have peculiarly shaped ascospores in which it is wise to describe germination. In *D. globosus* the ascospore is round or globoid and enveloped in a warty wall. When

[1] Klöcker, A. Eine neue Saccharomyces Art (S. Saturnus). Comp. Rend. trav. lab. de Carlsberg, 6, 1903.

it germinates, it undergoes at first a swelling during which these warts disappear. (Klöcker.[1])

The ascospores of *Schw. occidentalis* have also a warty wall and are divided into unequal parts. The largest of these possesses a projection thread. At the time of germination, the smallest part of the ascospore, that which does not possess the projecting portion, swells, loses its warts, and gives the impression that the ascospore possesses two walls. The larger part, that which does not undergo an enlargement, appears clothed with an outer layer which the ascospore tears when it grows. (Fig. 41.)

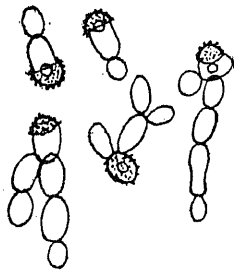


Fig. 41. — Germination of Ascospores in *Schwanniomyces occidentalis* (according to Klöcker).

Fifth Example, *Saccharomycopsis guttulatus:* The ascospores of this yeast are elongated and clothed, as has been stated, with two membranes, an endoplast and ectoplast. According to Wilhelmi[2] the germination begins with an enlargement of the ascospore, which causes a rupture of the ectoplast. (Fig. 42.) This rupture is accomplished at one end or on the side. Soon after budding begins and proceeds in the usual manner. During this budding, the ectoplast becomes irregular, shrivels up and leaves a little attached to the ascospore.

Sixth Example, *Monospora cuspidata* and *Nematospora Coryli:* These two yeasts are also characterized by ascospores with special shapes. In *Monospora cuspidata* the germination has been described by Metschnikoff.[3] The ascospores shaped like needles germinate laterally and in a prolonged form with oval buds. These break apart slowly.



Fig. 42. — Asc in *Saccharomycopsis guttulatus* Showing Two Ascospores at the Beginning of Formation. The exosporium is ruptured (after Wilhelmy).

In *Nematospora coryli*, in which the ascospores are fusiform and terminate, at one or both ends, in a long cilium, the disappearance of this cilium is soon accomplished and the ascospore assumes the shape of a short thick cell. These bud also at one or both ends.

Seventh Example, *Schizosaccharomyces:* With *Sch. octosporus*[4] the ascospores are able to remain in the interior of the

[1] Klöcker, A. Deux nouveaux genres des Sacch. Comp. Rend. des trav. lab. de Carlsberg, 8, 1909.

[2] Wilhelmi, A. Beiträge zur Kenntniss des *Sacch. guttulatus*. Inaug. Dissert. Bern. Lena, 1898.

[3] Metschnikoff, E. Ueber eine Sprosspilzkrankheit der Daphnien, Virchows Archiv, 96, 1884.

[4] Guilliermond, A. Observations sur la germination des spores du S. Ludwigii. Bull. Soc. de mycol. de France, 2, 1903.

asc, but very often the wall of the asc is absorbed and the spores are set free.

In the latter case, they may isolate themselves or remain united. At the time of germination, they commence to enlarge and become large cells similar to those in the vegetative stage. During this time the wall of the asc, if it exists up to this time, breaks up and is absorbed. But it often remains in the state of a veil during the partition of the ascospores. The ascospores sometimes remain spherical and form in the middle a wall which divides them into two daughter cells. These become round and separate. But more often they elongate. (Fig. 14, *a*.)

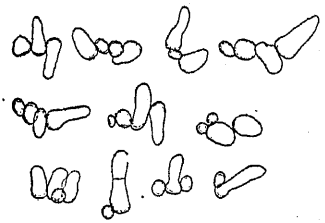


Fig. 43. — Germination of Ascospores in *Schizosaccharomyces Pombe*.

In *Sch. mellacei* and *Sch. Pombe* the ascospores germinate after the absorption of the membrane of the asc. This absorption is accomplished very quickly. They enlarge and each gives rise to a little tube which soon divides and forms two bacilli-like cells. Soon these, in their turn, divide in the same manner and furnish numerous generations of vegetative cells.

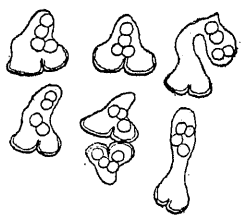


Fig. 44. — Abnormal Germination of Ascospores in *Saccharomyces Ludwigii*, on slices of carrot.

The zygospore resulting from the fusion of two ascospores changes directly into an asc.

Direct Germination of Ascospores in Asc: The investigations of Hansen and Guilliermond have shown that under certain conditions budding may be suppressed and that the ascospores, after becoming enlarged, are susceptible to germination without undergoing multiplication. This produces, then, a curious shortening of the development.

Hansen [1] has observed this phenomenon in *Saccharomyces cerevisiae* and the yeast Johannisberg II in the following manner. He placed some ascs of this yeast in beer wort in a Freudenreich flask. At the end of two hours, the ascospores enlarged and often copulated. After from three to five hours, the wall of the asc broke and the ascospores grew larger and larger. He placed some others in Freudenreich flasks containing a saturated solution of calcium sulfate. (It will be seen further on that calcium sulfate has the property of arresting immediate budding.) Under these conditions the ascospores are not able to germinate by budding and immediately go into ascs.

[1] Hansen, E. C. Recherches sur la physiologie et la morphologie des ferments alcooliques. Comp. Rend. des trav. lab. de Carlsberg, 5, 1902.

Guilliermond[1] accidentally observed the same phenomenon in the same yeast and in others (*S. Ludwigii, Willia Saturnus*) by a procedure much more simple, by making the yeast ascospore germinate on slices of carrot. In this nutrient medium, the ascospores germinate very rapidly and produce numerous generations of vegetative cells. But at the end of a few days, the multiplication is arrested, probably by an accumulation of toxic substances which may play a rôle similar to the chalk. The cells are then caused to sporulate. But as the majority of ascospores germinate immediately in this medium, others, less vigorous, do not begin to germinate until the vegetative cells produced by the germination of the first begin to sporulate. Under these conditions, germination of these tardy ascospores is without doubt restrained by the presence of toxic substances, secreted by the vegetative cells. Thus they are not able to bud nor be transformed into asci. With *S. Ludwigii*, for example, one may see fused ascospores which, with an enlarged copulation canal, produce new ascospores inside. We have formed, in this way, two swellings connected by an isthmus and resembling very closely an asc of *Zygosaccharomyces* or *Schizosaccharomyces*. (Fig. 44.) Sometimes the ascospore attempts to germinate and produces a tube for germination which, not being able to complete its development, enters the asc stage.

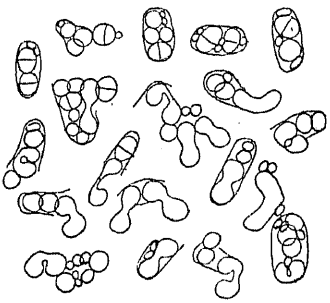


Fig. 45. — Abnormal Germination of Ascospores of *Schizosaccharomyces octosporus* on slices of carrot.

The ascospores fuse two by two, producing ascs immediately, or soon form cross walls producing cells to make ascs.

Guilliermond has found the same thing in *Sch. octosporus*. Here, the ascospores are able to fuse two by two and form an egg which soon is transformed into an asc. Often, they undergo one or two divisions, the daughter cells fusing to produce new ascs. (Fig. 45.)

This direct germination of ascospores in the ascs is explained easily by the fact that the ascospores have the import of a vegetative cell. It may be able to sporulate when conditions are favorable and may not have need to undergo a preliminary multiplication.

[1] Guilliermond, A. Observations sur la germination des spores du *S. Ludwigii*. Bull. Soc. de mycol. de France, 2, 1903.

CHAPTER II

CYTOLOGY OF YEASTS

General Considerations. Historical

FOR many years the cytology of yeasts has been concerned with the nucleus. Do yeasts have nuclei like other organisms? Or, on the contrary, are they deprived of a nucleus and therefore an exception? The question of a nucleus in the yeasts has given rise to a great number of reported investigations which allow contradictory conclusions. Some authors, among others Dangeard, Janssens, LeBlanc, Bouin, etc., have described bodies in yeasts which seemed to them to be nuclei; but others, having noticed a great number of disseminated particles in the cells, have admitted the presence of a "Diffused Nucleus." They believe that the chromatin is more or less mixed with the protoplasm of the cell and sometimes condensed in the form of colored grains. Eischenschitz, having noticed that these grains were particularly abundant in the vacuole, admitted that this last was a sort of rudimentary nucleus.

This conception was specified by Wagner[1] in 1898 and again by Wagner and Peniston.[2] These authors described in the yeasts, first, a vacuole, *vacuole nucleare*, filled with chromatin particles, and secondly, a nucleole of homogeneous appearance, situated at the exterior of this vacuole but always close to it. The whole of this vacuole is filled with particles of chromatin and, according to these authors, is a rudimentary nucleus representing a primitive step in the phylogenetic development of the nucleus.

Guilliermond[3] has given this debated question of yeast structure much study since 1901. It has been shown that the interpretation of Wagner is inexact, and the yeasts have a structure identical with

[1] Wagner, H. The nucleus of the yeast plant. Ann. of Botany, 12, 1898.

[2] Wagner, H., and Peniston, A. Cytological observations on the yeast plant. Ann. of Botany, 24, 1910.

[3] Guilliermond, A. Recherches sur la structure de quelques champignons inférieurs. Comp. Rend. Acad. des Sciences, 133, 1901. Recherches histologiques sur la sporulation des levures. Comp. Rend. Acad. Sci. 133, 1901. Recherches cytologiques sur la levure. Thesis for Doctor of Science, Paris, 1902. Remarques sur différentes publications parues récemment sur la cytologie des levures, Cent. Bot. 26, 1910. Nouvelles recherches sur la cytologie des levures. Comp. Rend. Acad. Sci. 150, 1910.

that of other fungi cells with a perfectly characterized nucleus. These investigations have shown that the body which Wagner took for a nuclear vacuole is in reality another thing — simply a vacuole with metachromatic corpuscles. In contradistinction to the nucleus of Wagner, it is not homogeneous. The existence of a nucleus cannot be doubted. The presence of it is now admitted.

Let us now investigate with detail the different elements which make up the yeast cell, that is, the nucleus, cytoplasm, the elements which it contains, and finally the cell membrane. Then let us review the phenomena which evolution has accomplished in the cell.

The Nucleus

The nucleus is relatively large in comparison to the cell (about 1 μ in diameter). It occupies a variable position, depending upon the form of the cell and its stage of development. Whatever its location, it is often closely associated with the vacuole which encloses the metachromatic granules. This is easily explained by the fact that the nucleus seems to play a rôle in nutrition and secretion, and that the vacuole is the seat of an intense secretion.

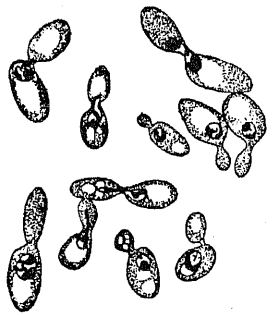


Fig. 46. — *Saccharomyces ellipsoideus* with Its Nucleus.

From a Preparation Stained with Hematoxylin.

The nucleus almost always presents a well-differentiated structure. It is surrounded by a colored membrane, filled with a colorless interior in which are a nucleolus and a chromatic framework more or less abundant and visible, depending upon the species. (Fig. 46.) In *S. cerevisiae* this chromatic framework is very distinct, the chromatin being particularly abundant. By its structure this nucleus is not distinguishable from the nucleus of other organisms, notably those which are present in most of the fungi. Today the nucleus is unique even in those cells which are elongated and which tend to form the rudiments of a mycelium. Later on we shall take up nuclear division.

The Cytoplasm and Its Different Products

The cytoplasm undergoes, as will be seen in connection with the evolution of cells, great variations in the course of development. Very dense and homogeneous in young cells, it encloses in the majority of yeasts, especially the spherical or oval yeasts (*S. cerevisiae, ellipsoideus, Pastorianus,* etc.), a vacuole filled with corpuscles

(nuclear vacuole of Wagner). Sometimes in the long yeast cell (*S. Ludwigii, Sch.* - *Pombe* and *mellacei, Mycoderma*) it possesses two such vacuoles situated at both ends of the cell and separated by a sort of very dense cytoplasmic bridge in which the nucleus is situated. (Fig. 47, 5.) In the course of development other vacuoles may appear at the side of these and include glycogen, giving the cell an alveolar appearance. At the same time, the cytoplasmic structure which limits these vacuoles is filled with numerous grains of various forms and sizes which are colored in the same manner as the nucleus which we have called "basophile grains." Finally, droplets of fat are also often seen.

The cytoplasm is then the seat of numerous secretions: metachromatic granules, glycogen, basophile grains, and fats. The characters of these special products will now be taken up.

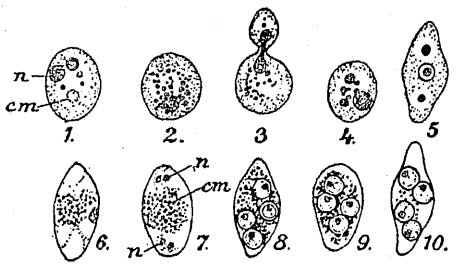


Fig. 47. — Yeast Cells with Nuclei (*n*) and Metachromatic Corpuscles (*cm*). From a Stained Preparation.

1–4, *S. cerevisiae*, beginning of fermentation; 5, *S. Ludwigii*, beginning of fermentation; 6–10, evolution of metachromatic corpuscles during sporulation.

A. Metachromatic Granules: The metachromatic granules, which were first studied by Guilliermond,[1] constitute the most important elements which are found in yeasts. They seem to play a very important rôle in cellular life. These bodies are almost exclusively localized in certain vacuoles, be it in a regular vacuole occupying the middle of the cell or in two polar vacuoles. They are able to exist also in the cytoplasm which surrounds the vacuoles. It is there that they seem to originate elaborated by cytoplasm and probably with the participation of the nucleus, because it is almost always in contact with the vacuoles. Once elaborated by the cytoplasm, they localize in the vacuoles, at whose expense they enlarge, in order to eventually dissolve at the time of their utilization. The metachromatic corpuscles are easily visible in living cells where they appear as refractive particles in the vacuoles, and seem to possess a Brownian movement. They may be fixed in the living condition by such dyes as methylene blue, neutral red, toluidin blue, etc.

[1] Guilliermond, A. Recherches sur la structure de quelques champignons inférieurs. Comp. Rend. Acad. Sci. 133, 1901.

Guilliermond, A. Recherches histologiques sur la sporulation des levures. Comp. Rend. Acad. Sci. 133, 1901.

Guilliermond, A. Recherches cytologiques sur les levures et quelques moissures à forme levures. Thesis for the Doctorate at the Sorbonne, Storck, exit. Lyon, 1902.

The investigations of Dangeard have shown that the metachromatic granules are produced from a condensation of a metachromatin matter existing in the vacuole in the state of a colloidal solution. In the living cell they are rather numerous, but staining brings out larger numbers. The metachromatin precipitates under the influence of the vital stains. It acts the same way towards fixatives.

After fixation by alcohol, the granules are stained more deeply than the nucleus by the nuclear stains. They take the colors of the basic aniline blue and violet dyes and assume a color between a red and violet. With hemotoxyline or hematine they are colored a wine red. This metachromatism, to which they owe their name, distinguishes closely between the nucleus and other bodies in the cell.

The metachromatic corpuscles are present in great abundance not only in yeasts but also in many of the Protista. We have shown that they are identical with other bodies which have been observed formerly in the bacteria and *Cyanophyceae* by Babes and Bütschli, and regarded as grains of chromatin. Bütschli has called them "Red granules" on account of their metachromatism and we have retained by reason of its priority the term "metachromatic granules" suggested by Babes. This ought to be used also instead of the term "grains volutine" proposed by A. Meyer.[1] The metachromatic granules have been pointed out, since, in the fungi, algae, and protozoa. On the contrary they do not seem to be present in the *Metazoa* or the *Metaphytes*. According to the observations of A. Meyer and Guilliermond, in collaboration with Beauverie,[2] the globoid grains of the Phanerogames contain, associated with glycerol or saccharine phosphates, a nitrogenous substance which seems to be much like the substance which makes up the metachromatic granules. This is metachromatin, more or less like that which is found in yeasts. The granulations of Mastzellen or leucocytes present histo-chemical properties, much like those of metachromatin, as has been pointed out by the investigations of Guilliermond[3] and Mawas.

There is, then, sufficient evidence for considering the metachromatin as composed of nucleic acids. The recent investigations of van Herwerden have given good reasons for favoring this hypothesis. By cultivating yeasts in media completely deprived of phosphates, this author has noticed that these yeasts never contain the least

[1] Meyer, A. Orientierende Untersuchungen über Verbreitung, Morph. und Chemie des Volutins. Bot. Zeitg. 62, 1904.

[2] Beauverie, J., and Guilliermond, A. Note préliminaire sur les globoïdes. Comp. Rend. Acad. Sciences, 1906.

[3] Guilliermond, A., and Mawas, J. Caractères histo-chimiques des granulations des Mastzellen. Comp. Rend. Acad. Biol. 64, 1908.

trace of metachromatic granules in their cells. On the other hand, by cultivating these yeasts which have been deprived of their granules in media with phosphate, van Herwerden has noticed the immediate appearance of metachromatic corpuscles. A nucleic acid compound is extracted, along with volutin or metachromatin, by dilute alkali from *Torula monosa* and *Saccharomyces cerevisiae*. This cannot be obtained from an equal quantity of volutin-free culture. This seems to prove what has been indirectly supported in the past, that metachromatin is made up of a nucleic acid compound. No doubt obtains but that the nucleic acid from yeast originally came from the volutin. This nucleic acid is decomposed by a nuclease formed in the *Torula* cells, in which process the formation of phosphoric acid could be demonstrated. The metachromatin free cultures also contain a nuclease. This is contrary to the opinion of Henneberg who claims that the metachromatin is the enzyme itself. This substance, according to van Herwerden, is probably a nucleic acid and possibly a reserve material. While it may not be indispensable for the growth of the cells it may be of importance in their individual development. It may be related to the fermenting ability by supplying small amounts of phosphates[1] which may be liberated from the nucleic acid by the nuclease.

The metachromatic corpuscles are certainly nitrogenous products, but their exact chemical nature is not completely known. However, after the investigations of Meyer, Kohl,[2] and Reichnow,[3] it is admitted that they result from a combination of nucleic acids. Kohl regards them as nucleoproteins. Meyer has demonstrated that the histo-chemical reactions of metachromatin resemble those of nucleic acid and that there are other organisms which chemical analysis reveals to have more nucleic acid, as the yeasts and certain bacteria, which contain more chromatin. Kossel has been able to isolate from yeasts a large amount of nucleic acid, and this seems disproportionate to their relatively larger nucleus. It is probable that a greater part of this nucleic acid comes from chromatin. Reichnow[3] has demonstrated that in *Haematococcus pluvialis*, which normally contains much metachromatin, this substance disappears and does not re-form when the alga is cultivated in a medium entirely devoid of phosphorus. Nucleic acid is especially rich in phosphorus. On the other hand the researches of Giemsa seem to indicate that

[1] The investigations of Levene and Kossel have indicated the presence of large amounts of phosphoric acid in yeasts.

[2] Kohl, G. Hefepilze, Leipzig, 1908.

[3] Reichnow, E. Untersuchungen an Haematococcus pluvialis. Arb. a. d. Kaiserl. Gesundheitsamte, 33, 1909.

the affinity of the nucleus for dyes depends upon the content of metaphosphoric acid. Perhaps, with a little reservation, we may explain the staining properties of the metachromatin in the same manner.

With regard to the rôle of the metachromatin, our knowledge is happily more complete. Certain bacteriologists have tried to connect the pathogenicity of bacteria with their content of granules. They have regarded these as the toxic products of the bacteria or, more definitely, as products initial to the secretion of toxins. This theory was supported by Behring,[1] who pretended to have extracted the metachromatin from *Bacterium tuberculosis* and stated that this substance corresponded to the toxin of that Bacterium. According to him a gram of this substance on the dry basis will be as toxic as a liter of Koch's tuberculin. It is probable that the metachromatin isolated by Behring was not in the pure state. The investigations of Guilliermond have indicated that the metachromatin has no relation to toxins, and that it ought to be regarded as a reserve product. The metachromatic granules are quite abundant during periods of great vital activity in the yeasts. They diminish and finally disappear in old cultures. They disappear very quickly in yeasts undergoing inanition.

Henneberg[2] has attempted to show that the metachromatic granules are related to fermentation. According to this investigator, it is during the period of greatest fermenting activity that these bodies are most highly abundant in the yeast cell. This is also accompanied with an increase in metachromatin (volutin). The addition of phosphates, which caused a great increase in the fermenting ability of the cells, also caused an increase in metachromatin. Henneberg thinks that metachromatin is the zymase itself. Since these metachromatic corpuscles are found in all fungi, Henneberg states that metachromatism may be a general reaction for a certain group of enzymes, just as the guaiac reaction is characteristic for all oxidases. This theory is almost untenable.

The rôle of the metachromatin explains itself when the sporulation of yeasts is studied. It has been stated that the metachromatic corpuscles accumulate in yeast cells destined to sporulate. They dissolve, and following this, are absorbed by the ascospores, and disappear entirely in the maturity of these cells. (Fig. 47.) They undergo the same evolution as the fats and glycogen, which are

[1] Behring. Congrès de la tuberculose, Paris, 1906.

[2] Henneberg, Volutin of the yeast cell. Woch. Brau. 32 (1915), 301–4, 320–3, 326–9, 335–7, 345–7, 351–4. On the volutin (metachromatic granules) in the yeast cell. Cent. Bakt. Abt. II, 45 (1916), 50–62.

very abundant in the cells about to sporulate, and play, like them, the rôle of reserve material. The results which have been secured by Guilliermond[1] on the evolution of the metachromatics in the higher ascomycetes and various molds have confirmed this opinion. In the young ascs of the higher ascomycetes, the many metachromatic granules collect about the ascospores in formation when they are finally absorbed by the ascospores. In the molds (*Penicillium, Sterigmatocystis*) they accumulate in the fruiting heads and serve in the nutrition of the conidia. Van Herwerden admits that these bodies represent reserve products which will be decomposed by a nuclease with the formation of phosphoric acid, and this favors the fermentation.

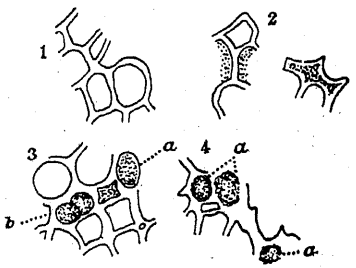


Fig. 48. — Yeast cells with a formation of mucilaginous network. The network has been obtained by partial drying.

1, Part of the cells has fallen. In 2 and 3 it is seen that the network may affect the form of the entire wall, for example between *a* and *b*; *a* is a vegetative cell; *b* an asc with two ascospores; 4 *a*, three cells living in the network (according to Hansen).

According to Amata,[2] two kinds of lipoidal granulations may be demonstrated in the yeasts by Soudan III. Some resist the fat solvents after treatment with organic acids and become blackened. The others become a brownish color after treatment with organic acids and are dissolved in xylol and ether. The first type is less abundant than the second type.

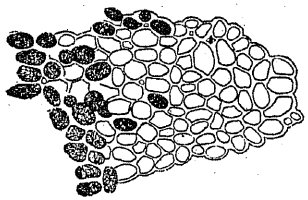


Fig. 49. — Formation of Network and Yeast Cells. The Latter have been Colored with Methyl Violet. The Network is Uncolored. Some Cells are still Found in the Meshes, but Most of them are Removed (after Hansen).

B. Glycogen: Glycogen was observed for the first time in yeasts by Errera.[3] It is very abundant in the cells. It is easily recognized by the brown color (mahogany) which it gives with iodin in potassium iodide. The color disappears when the solution is heated to 60°, but reappears when it cools. Glycogen exists in almost all of the yeasts; however, certain species do not contain it at any moment in their development, perhaps because it is used up as soon as it is formed. In this category, belong *S. apiculatus, exiguus*, and the *Schizosaccharomyces*. On the contrary, we

[1] Guilliermond, A. Contr. à l'étude de la formation asques et de l'épiplasm des Ascomycètes. Rev. gén. de Bot. 14, 1903.

[2] Amata, A. Ueber die Lipoide der Blastomyceten. Cent. Bakt. Abt. II, 42, 1915.

[3] Errera, L. L'épiplasm des ascomycètes at le glycogène des végétaux. Thesis d'agrégation des sciences, Brussels, 1882.

have seen that the ascospores of the *Schizosaccharomyces* contain some starch which collects in the wall. This substance replaces glycogen and is used as a reserve during germination of the spores. Glycogen appears in the cells from the beginning of fermentation and reaches its maximum after 48 hours. It is almost always localized in the vacuoles distinct from those which contain the metachromatic granules. It diminishes gradually and disappears entirely towards the end of fermentation. During sporulation it accumulates in great quantities in the ascs and is absorbed by the ascospores during their maturity.

C. Basophile Granules: These granules, very rare in young cells, become very numerous in course of development, especially between 12 and 24 hours. (Fig. 50, 5 and 8.) They are not visible in the living cells and do not take stains. For the most part, they resist fixation and present somewhat the same color characteristics as the chromatin. They are stained especially by hematoxylin which gives them an intense black color like the nucleus. This is less resistant and they are easily decolorized. With the other nuclear stains, they differentiate themselves less closely from the nucleus. These granules offer variable shapes and dimensions. Many are angular, and certain authors, as Hieronymus and Kohl, have regarded them as crystalloids of protein. A close examination, however, reveals that they are not crystalline.

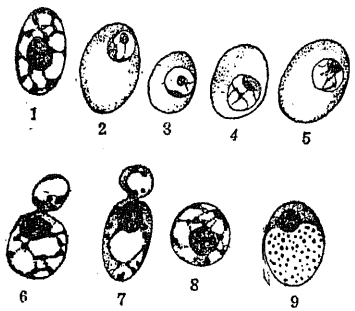


Fig. 50. — *Saccharomyces cerevisiae* in a Preparation Colored with Ferric Hematozyline.

1 to 4, beginning of fermentation; 5 to 8, between 12 and 24 hours; 9, after 48 hours.

The basophile grains are probably albuminoid substances, but it is not possible to state precisely their rôle. They are in all cases substances of nutrition (reserve materials) and do not seem to have a relation to fermentation, because they appear as well in yeasts cultivated under aërobic conditions, as in yeasts in the process of fermentation. On the other hand, they are numerous at the moment of sporulation and contribute to the formation of the ascospores.

D. Fats: These are present in the living cells under the form of refractive granules of variable size, situated in the cytoplasm, and are stained brown with osmic acid. Will has been able to color them red by means of tincture of alkanna and has brought about their dissolution by ether. Very abundant at the beginning of development in certain species (*Debaromyces globosus, Sch. occidentalis, Torulaspora, Torula*), these fat globules are absent or rather widely distributed in other yeasts, especially the industrial varieties. In

the majority of yeasts, they appear especially during sporulation and serve as food for the ascospores. They are, then, reserve products. Fat globules are also observed in old cells, but in this case seem to be due to a degeneration of the cytoplasm.

The Membrane

The membrane of the yeasts is thick and presents a double layer quite distinct. With the exception of *S. granulatus*, it is provided with isolated granulations which are placed in regular fashion. We have seen also that the ascospores of certain yeasts may offer a warty membrane. The chemical constitution of the membrane is only slightly known.

According to chemical analyses of Schlossberger, it contains a special cellulose which resembles fungine or metacellulose, and is distinguished from the true cellulose by its insolubility in ammoniacal cupric oxide, and reacts differently toward iodin.

Liebermann and Bitto in treating yeasts successively with acids and alkali obtained a cellulose which gave the zinc chloride reaction.

According to Salkowski, this cellulose is colored brown by iodin. Meigen and Spreng[1] claimed that they did not secure the true cellulose from yeast membrane but a hemicellulose which was easily hydrolyzed by the prolonged action of acids and alkalis. On the contrary, according to Will and Casagrandi, the membrane was not colored by iodin nor by the ordinary stains for cellulose. They found no cellulose. According to Casagrandi,[2] pectose makes up the membrane. Mangin[3] believed that it was composed of callose. Tanret and Visselingh found chitin in the membrane of beer yeast. Whatever is the case, the membrane stains blue with Ehrlich's methylene blue and Hanstein's aniline. This membrane is especially visible in the durable cells in which it thickens considerably.

According to Will and Casagrandi, the membrane of the durable cells is made up of two layers. (Fig. 10.) When the yeast is treated with 4 or 5 per cent hydrochloric acid, washed and dried, and stained with fuchsin according to the method of Strasburger, the outer layer takes a violet red color which is surrounded by a colorless layer.

Yeasts secrete under certain conditions mucilaginous substances which collect the cells into a sort of network quite similar to zoogloea. Hansen first attracted attention to this phenomenon which

[1] Meigen and Spreng. Ueber die Kohlhydrate der Hefe. Zeitschr. Phys. Chemie, 55, 1908.

[2] Casagrandi, O. Saccharomyces ruber. Ann. d'Igi sperim. 7 and 8, 1898.

[3] Mangin, L. Observations sur la constitution de la membrane chez les Champignons. Comp. Rend. Acad. des Sciences, 107, 1893.

appeared to play a rôle in the coagulation of yeasts, followed by a clarification of the liquid. This is comparable to the agglutination which is found among the bacteria. Hansen has obtained the production of a mucilaginous network by placing brewery yeast in a covered bowl and letting it stand while it slowly dries. When a portion of this yeast was examined in water, the formation of an entangling network was observed. (Fig. 48.) Similar formations are observed in yeasts placed on gypsum blocks or on gelatin. Hansen has observed the same phenomenon in cells from scums yeasts of certain species. This network is brought out especially when the cells are stained with methyl violet or methylene blue.

Certain pathogenic yeasts protect each cell by means of a thick capsule which is mucilaginous in nature.[1] Certain yeasts seem to unite with one another in a constant manner by means of a gelatinous substance. Lindau has observed this in yeasts which he has studied.

Changes in the Cell during Fermentation

The structure of yeasts is easy to interpret at the beginning of development. During the active period of fermentation they become more complex. What complicates the subject at this moment is a very active secretive action. Like all secreting cells, they present a series of cytological phenomena in connection with the secretions.

Let us observe these modifications which are produced in the cells in the course of fermentation by taking *S. cerevisiae* as an example. At the beginning the cells possess a cytoplasm very dense and homogeneous, a nucleus situated at the side of the cell and a vacuole filled with metachromatic corpuscles which occupies the center. (Fig. 47, 1 and 4, and Fig. 50, 1 and 4.)

After 24 hours of fermentation the cell undergoes very impor-

[1] Agglutination or flocculation of yeasts is a complex phenomenon which is little known. It seems to be brought about by a change in the constitution of the membrane which becomes viscous. It appears in connection with the formation of a gelatinous network described by Hansen. This is the agglutination to which one attributes the clarification of wine after fermentation. Beijerinck (Die Erscheinung der Flokenbildung oder Agglutination bei Alkoholhefen, Cent. Bakt. 20, 1908) distinguished autoagglutination, produced by the yeast itself, and symbiotic agglutination, brought about by bacteria developing at the same temperature as the yeasts (especially by the *Leuconostoc agglutinans*). Agglutination is brought about by the addition of sulfuric acid, boric acid or 1 per cent of other acids. Microscopically, the cells do not show any alteration during the agglutination. Agglutination seems to be related to the life of the cells, for dead yeasts do not agglutinate. Van Laer has shown that borax causes the agglutination of the yeasts killed by heat.

tant modifications. The cytoplasm is hollowed out by a certain number of little vacuoles which are distinct from the vacuole containing the metachromatic corpuscles. The cytoplasm, then, takes an alveolar structure. The nucleus always takes its place at the center; it seems to swell and take on an ameboid shape. One observes at this time in all of the cytoplasm, and especially about the nucleus and along the walls, a large number of basophile granules of irregular form, some angular and others filamentous.

After 48 hours fermentation, the glycogenic vacuoles fuse into a large vacuole which takes up almost all of the cell and absorbs the nucleus cytoplasm and the vacuole with the metachromatic corpuscles. The cell is then transformed into a sort of glycogenic sac. At this moment the glycogen seems to be retained by the cell, for it is not consumed. The income is greater than the expenditure. The basophile granules decrease in number and adhere to the wall of the cell. At this time, there appear in the glycogenic vacuole a considerable number of small granules which differ from the basophile grains by their smaller dimensions and lesser pigmentation and whose functions are unknown. At this stage the nucleus undergoes a variation in pigmentation which is very close; it stains intensely and takes on a homogeneous aspect. At the end of the fermentation, the cells assume the structure which they had at the beginning.

These are the modifications through which yeasts pass in the course of fermentation: change in the structure of the cytoplasm, appearance of grains of secretion, variation in pigmentation of the nucleus are the well-known phenomena in secreting cells. For the most part the yeasts fit this scheme with a few differences in detail.

Cytological Phenomena during Vegetative Multiplication

A. Budding: We have already described budding and it will not be necessary to recapitulate at this time. Let it suffice simply to indicate the cytological phenomena which take place during this change. By its appearance, the bud is made up of a very dense cytoplasm containing a few basophile grains which have emigrated from the mother cell. When it has acquired a certain dimension, a little vacuole appears in the midst of the cytoplasm which is filled with metachromatic corpuscles. This vacuole results often from the entrance of a little of the vacuole from the mother cell.

During this phenomenon, the nucleus occupies its usual position even if it is at the opposite end from the bud, and undergoes no modification until this has acquired its definitive dimension. At

this moment only, the nucleus, without changing its location, elongates and takes the shape of a dumb-bell. One of the heads of this enters the bud. (Figs. 46 and 47.) A separation then takes place which frees the two heads from one another. One part remains in the mother cell while the other is in the bud. Both nuclei thus formed retain for some time the shape of a club before assuming their normal appearance. The nuclear division does not offer the characteristics of karyokinesis contrary to the opinion of other authors (Swellengrebel[1] and Fuhrmann). It seems to consist simply of a direct division.

B. Transverse Division: Division is not observed as we have seen in the *Schizosaccharomyces.* It consists simply in the formation in the middle of the cell of a transverse partition which separates the two daughter cells. During this phenomenon, one may observe at both extremities of the cell, the formation of a little vacuole, since the nucleus situated in the center elongates into a dumb-bell, both heads of which are placed at ends of the cell. The middle connecting link is severed and the two heads form the nuclei of the two daughter cells which are to be separated by a transverse wall.

Cytological Phenomena of Sporulation

We have seen in the preceding chapter that a certain number of the yeasts possess sexual processes which function either at the moment of sporulation or germination of the ascospores. In the first case (*Schizosaccharomyces, Zygosaccharomyces, Debaromyces*) the asc results from the isogamic copulation of two cells. In the second (*Saccharomycodes, Willia saturnus*) copulation is effected between two ascospores at the time of their germination. We have been able, in order to present clearly, to describe by anticipation the phenomena (nuclear and cytoplasmic fusion) which take place during this copulation. We shall not repeat here.

With the exception of these species, none of the yeasts present any trace of sexuality; with them the asc forms at the expense of each cell without preliminary copulation.

It has been stated that the sporangium of yeasts is similar to the asc of the *Ascomycetes,* especially that of *Exoascees.* However, a difference exists between the asc of yeasts and the organ of the same name in the *Ascomycetes.* In all of the *Ascomycetes* which do not copulate at the moment when the asc forms, especially the *Exoascees,* this organ includes by its origin two nuclei, and it is only after the fusion of these two nuclei that it assumes its definite volume

[1] Swellengrebel, H. La Division nucléaire dans les levures pressées. Ann. Institut Pasteur, 19, 1905.

and forms ascospores. From recent studies [1] it has been shown that in the formation of the asc, especially in the *Exoascees*, which constitute a family of the *Ascomycetes* very close to the yeasts, this fusion is wanting.

In many yeasts which do not represent sexuality, and among others in *Saccharomyces cerevisiae*, Janssens and Leblanc [2] considered that they observed a nuclear fusion in the cells which were about to form ascs and considered this as a sexual act. According to these authors the nucleus of these cells undergoes a division since both nuclei, which result after being separated for some time, blend together into a single nucleus which by successive divisions furnishes the nuclei for the ascospores. But Guilliermond [3] has shown that this observation is inaccurate and that one is not able to verify any nuclear fusion in the yeast cells which are destined to sporulate without preliminary copulation. Thus karyogamy preceding sporulation is lacking in the yeasts, a fact which seems definitely established today.

We shall stop now to observe the processes which take place in the cell when it transforms into ascs — the division of the nucleus and the formation of ascospores.

Let us take *S. Ludwigii* as the example. In this yeast, sexuality before the formation of the asc has not been observed. As we have said, the cells pass directly into ascs without preliminary copulation and this phenomenon is replaced by a fusion (parthenogamy) of the ascospores at the moment of germination.

The cells which are preparing to sporulate assume a very complex structure. They possess an alveolar cytoplasm in which the network which surrounds the alveoli shows inclusions of fat and numerous basophile granules. Two sorts of alveoli may be distinguished: some are filled with a considerable quantity of metachromatic corpuscles; others with glycogen. The nucleus is placed at the side of the cell. It surrounds itself with a thin layer of very dense zone of protoplasm (plasma sporogenic) at the expense of which the ascospores are built up. In this sporogenic plasma, the greater portion of the basophile grains accumulate. (Figs. 47, *a*, and 51.) All the rest of the cytoplasm, which possesses an alveolar structure, will not be used in the formation of ascospores, but will form the epiplasm, the plasma which will be absorbed by the ascospores during their

[1] Dangeard, who observed this nuclear fusion first, regarded it as a true fecundation. The explanation of this phenomenon is not completely elucidated and remains obscure.

[2] Janssens, F., and LeBlanc, A. Recherches cytologiques sur la cellule de levure. La cellule, 14, 1898. A propos du noyau de la levure. La cellule, 20, 1903.

[3] Guilliermond, A. Le noyau de la levure. Annales mycologici, 2, 1904.

maturation and serve them as food. At this stage, which corresponds to the beginning of nuclear division, some important modifications appear in the alveoli which contain metachromatic corpuscles. These bodies, increased in number and diminished in volume, undergo a sort of pulverization which reduces them to infinitely small particles which, in their turn, dissolve, the alveoli taking the same color that pertained to the corpuscles. This phenomenon of dissolution of the metachromatic granules is followed by the formation of the ascospores.

During this time the nucleus undergoes its first division; but, the very chromophile cytoplasm, filled with basophile grains which surround it, does not allow the division to be followed nor any knowledge with regard to how it operates. One is able to see only two small nuclei closely related to each other and situated in a zone of sporogenic plasma, which advance by steps to a single large nucleus. However, certain aspects of the phenomenon seem to indicate that the nucleus divides by karyokinesis. This has been put in evidence for *Schizosaccharomyces octosporus.*



Fig. 51. — Formation of Ascospores in *S. Ludwigii.*

The two daughter nuclei soon emigrate to both poles of the cell. They are followed by the sporogenic plasma which divides between the two poles in order to surround the nuclei. At this moment, one may observe the steps in which there are two nuclei at each end of the cell surrounded by a zone of sporoplasm and separated by a portion of the same material. Some authors (Janssens and Leblanc) have attributed this to sort of karyokinetic structures, regarding the two nuclei surrounded by sporoplasm as the anaphase plates and the thread plasma which unites them as an achromatic spindle.

Soon the thread which unites the two masses of protoplasm disappears and each nucleus undergoes a division. We have seen, then, the stages in which two small nuclei are placed one at each pole of the cell with a mass of sporoplasm about each. (Figs. 47, 7, and 51.) The sporoplasm concentrates about each and forms 4 little balls provided with a nucleus and placed in pairs at each pole. These are the ascospores. These increase in size and form a membrane about them. At this moment the epiplasm becomes disorganized and is reduced to an alveolar fluid containing fats, glycogen and metachromatic corpuscles. The metachromatic granules slowly

disintegrate and are finally ingested by the ascospores. Little by little they disappear entirely, being absorbed by the ascospores during their development. (Fig. 47, 10.) The glycogen and the fats undergo the same fate and are absorbed by the ascospores. A part of these different products is consumed by the ascospores, the other is kept in reserve in the ascospores to serve during germination. The ascospores increase little by little in size and eventually occupy the entire volume of the asc, after having absorbed the entire epiplasm with its metachromatic corpuscles, fats, and glycogen.

The ascospores reach the adult state presenting a thick membrane and a central nucleus from which the cytoplasmic rays containing fats start, and which delimit small vacuoles. These include a little glycogen and a few corpuscles, products which will be consumed at the moment of germination.

In all of the yeasts, the cytological phenomena are the same differing only in details. In *Saccharomyces cerevisiae, ellipsoideus,* and *Pastorianus,* instead of forming at two poles, the ascospores originate generally in the middle of the cell in a zone of sporoplasm. They undergo one or two divisions depending on the number of ascospores, and the nuclei which result remain very close to each other in the same zone of sporoplasm which soon concentrates about each of them to make up the ascospores.

In the *Schizosaccharomyces* and especially in *Sch. octosporus,* the asc contains many less metachromatic corpuscles and basophile grains than in other yeasts; also the cytological phenomenon of the formation of the ascospores is much easier to observe. After the nuclear fusion, the nucleus grows and soon undergoes a first division. Guilliermond [1] has shown that this is a karyokinesis similar to that described among the *Ascomycetes.* It is almost always accomplished in the direction of the long axis and is manifested by the presence of an achromatic spindle made up of small granular particles more or less distinct which represent the chromosomes of the equatorial plate. The chromosomes then distribute themselves along the spindle. At this moment, the nuclear membrane seems to be absorbed while the spindle elongates in such a manner as to form granular masses at each end of the cell. The nucleolus persists at the side of the spindle, but finally disappears. Two nuclei are thus formed which go to different parts of the cell. The two daughter nuclei which result emigrate to both extremities of the cell to undergo another division and sometimes a third, from which result 4 or 8 ascospores. The nuclei thus formed are disseminated in the cytoplasm, which has

[1] Guilliermond, A. Sur la division nucléaire des levures. Annales de l'Institut Pasteur, 31 (1917).

an alveolar structure. Each of these surrounds itself with a small zone of dense protoplasm, and then transforms itself into ascospores. These grow at the expense of the cytoplasm until they occupy the whole asc.[1]



Fig. 52. — Germination of Ascospores in *S. cerevisiae.*

The cytological phenomenon of the formation of ascospores presents many characteristics in common with that observed in the ascs of other *Ascomycetes*, especially the endomycetes.

The germination of ascospores when they are not accompanied by a copulation, does not offer any special characteristic. The ascospores in time swell up and are transformed into vegetative cells which bud after the normal procedure. (Fig. 52.)

[1] Beauverie has proposed a method for staining ascospores. The yeast should be fixed in alcohol or formol and stained with carbol fuchsin heating to the point where vapor is given off. It should then be decolorized by 1–3 acetic acid, washed in water and stained by thionine. The spores will be stained red and the rest of the cell blue. This ability to resist acids is especially marked in *Schizosaccharomyces octosporus*. Beauverie, J., Quelques propriétés des ascospores de levures. Technique pour leur différentiation. Comp. Rend. Soc. Biol. 80 (1917), 5.

CHAPTER III[1]

PHYSIOLOGY OF YEASTS. NUTRITION, RESPIRATION, AND ALCOHOLIC FERMENTATION

YEASTS are able to undergo two very different kinds of life. Sometimes they live in contact with air and respire — under aërobic conditions; at other times, in the absence of air. In this latter case, they take the energy which is necessary from another process. They transform the greater portion of sugar at their disposal into alcohol and carbonic acid. They thus induce an alcoholic fermentation, which is then anaërobic. One must distinguish between the yeast plant which acts like other ordinary plants and the yeast ferment which is the agent of alcoholic fermentation.

We shall take up in this chapter the general nutritive processes of the yeast, that is, its nutrition, respiration, and alcoholic fermentation, reserving for a following chapter the study of the relation of yeasts to their external environment, of the conditions which determine their multiplication, sporulation, and parasitism.

We shall begin by investigating the chemical make-up of the yeasts and by studying the various enzymes which prepare foods for absorption.

GENERAL PHENOMENA OF NUTRITION OF THE YEASTS

Chemical Composition of the Yeasts

The different analyses of yeasts undertaken by various authors have given variable proportions of C, H, N, O, and S. (Dumas, Schlossberger, Mitscherlich.) Analysis of the ash of yeasts has given equally inconstant results. Phosphoric acid, silicic acid, carbonic acid, sulfuric acid, hydrochloric acid, potassium, sodium, sulfur, magnesium, calcium and ferric oxide and manganic oxide have all been found to exist in different proportions.

Thus, as we have seen in the preceding chapter, the yeast cell is composed essentially of a membrane which seems to be made up of

[1] In the preparation of this chapter, the obliging collaboration of M. A. Polecard, D.Sc., Chief of the Department of Physiology of the College of Medicine of the University of Lyon, is acknowledged.

cellulose or a closely related substance, of an albuminous protoplasm and a nucleus rich in nuclein. The yeasts contain hydrocarbons, albuminoids, and fatty bodies.

Let us consider successively these groups of substances.

Hydrocarbon Materials: The analysis of Schutzemberger has shown the presence of a substance like cellulose in the membrane, which seems to be formed from sugar, and of a gummy substance which seems to be transformed from this cellulose under the influence of the chemical agents used in its preparation. Finally Errera and Clautriau[1] have disclosed the presence of glycogen which, according to Laurent, is able to reach a concentration of 32 to 38 per cent.

Fatty Bodies: Fats to the extent of 5 per cent of the dry material have been reported. However, in old cells, the fat may increase to even 20 per cent. This is not surprising, for we have stated that it may exist in two forms: one as reserve products formed, without doubt, from the sugars (Pasteur); the other seems to come from a protoplasmic degeneration. The first is generally rare during fermentation and appears especially during sporulation. The other is observed in old cells in the state of degeneration.

The fatty materials of yeasts are generally acid in reaction and composed of ordinary fats, cholesterol, lecithin and phytosterol. The weight of cholesterol may reach 0.06 per cent of the dry yeast, according to Lowe, but increases in old cells. Hinsberg and Ross have pointed out the presence of an ethereal oil, not saponifiable, with a hyacinth odor to which he attributes the special odor of yeasts.

Welter[2] in discussing a yeast which contains 50 per cent of protein states that it contains 4 per cent of fat and this may be increased up to 17 per cent. It is thought that the fat is produced by a transformation of sugar. Bokorny[3] states that to secure abnormal fat formation in yeasts, they must be fed quantities of carbohydrates and proteins. From the data which he secured he regards the cell protein as the source of the fat. Neuss[4] reports a yeast which contained 18 per cent of fat on the dry basis. Under special conditions of cultivation this could be increased to 50 per cent. The fat was similar to olive oil.

[1] Clautriau, G. Études chim. du glycogène chez les champ. et les levures. Ac. roy. de Belgique, 3, 1895.

[2] Welter, A. Yeast fat, a new source of fat. Seifenfabriken, 35, 845–6, 1915. Chem. Abstracts, 10, 124, 1916.

[3] Bokorny, T. Yeast fat. Allgem. Brau-Hopfen Ztg. 55, 1803–5, 1915. Chem. Abstracts, 10, 798, 1916. Assimilation of fat in plant cells, especially in yeasts. Arch. Physiol. 1915, 305–49. Chem. Abstracts, 11, 2218, 1917.

[4] Neuss, O. Yeast fat. Seifenfabr. 36, 38, 1916. Chem. Absts. 10, 977, 1916.

Amato[1] demonstrated the presence of lipoids in *Saccharomyces cerevisiae* by chemical and microchemical methods. He treated yeasts in fixed preparations with osmic acid, finding that most of the granules became brown in color. By their reaction to fat solvents, the majority of these granules were regarded as belonging to Bernard and Bigart's labile fats. Amato thinks that most of the lipoids in yeast are lecithins. Extraction of both the washed and dried yeast with ether gave a residue which, after combustion, and extraction with sodium carbonate, yielded the characteristic phosphoric acid precipitate with ammonium molybdate. Bokorny[2] has reviewed this subject from the point of using this yeast fat commercially. The need for these fats was especially emphasized in Germany during the war on account of the successful blockade of German ports by the Allies. Bokorny stated that much study was required before the yeast fat could be put on a commercial basis. Neville[3] studied yeast fats and found that the principal fatty acids had the empirical formula $C_{15}H_{30}O_2$, but that arachidic acid, $C_{20}H_{40}O_2$, melting at 77° C., was not very abundant. Unsaturated acids could not be separated in the pure state. Oxidation with $KMnO_4$ yielded the corresponding di- and tetrahydroxy acids which indicated the presence of $C_{16}H_{30}O_{27}$, $C_{18}H_{34}O_2$ and $C_{18}H_{32}O_2$ in the fat. Cholesterol melting at 145–147° C. was obtained. Bokorny[4] has made further observations on yeast fat and found a greater accumulation when the source of nitrogen was peptone than when amino acids (glutamic and aspartic) were used. Dilute urine to which sugar had been added represented the cheapest source of nitrogen.

Albuminoids : A material approaching egg-albumin in characteristics has been found in yeasts. According to Trommsdorf and Meisenheimer[5] the cake obtained by the compression of the yeasts, in the preparation of yeast juice, which contains *zymase*, is colored black by Grams solution and safranin while the juice takes a red tint with the same reagents. There must be present, then, in yeast a soluble albumin which may be colored red and an insoluble albumin which takes a black color according to this procedure.

There has been proven in yeasts, an albuminoid substance soluble in warm alcohol, which must be a peptone produced by the action of

[1] Amato, A. The lipoids of blastomycetes. Cent. Bakt. Abt. III, 42, (1915) 689–98.

[2] Bokorny, Th. Yeast fat. Allgem. Brau-Hopfen Ztg. 55, (1915) 1803–5.

[3] Neville, H. A. D. The fat of yeast. Biochem. Jour. 7, 341–348.

[4] Bokorny, Th. Accumulation of fat in plant cells, especially in yeasts. Arch. physiol. 305–349, 1915; Chemical Abstracts, II, (1917) 260.

[5] Meisenheimer, J. Neue Versuche mit Hefepressafs. Zeit. physiol. Chemie, 37, 1904.

endotryptase, a proteolytic enzyme which we shall discuss further on in this book. Also derivatives of the albuminoids have been demonstrated such as the amino acids (leucine and tyrosine); these are also products of digestion by the endotryptase.

Schutzemberger [1] has demonstrated the purine derivatives (xanthine, hypoxanthine, guanine). Kossel [2] and Buchner [3] have found nucleic acids in rather large quantities.

As the nucleus of yeasts is usually small and poor in chromatin, Meyer has been led to think that this nucleic acid is not derived entirely from the nucleus but also from the protoplasm. According to this author, the metachromatic corpuscles which are so abundant in yeasts result, as we have said, from a combination of nucleic acids with an unknown organic base. Kohl agrees with this and also states that these bodies represent nucleo-proteins.

Belohoubek has analyzed yeasts chemically with the following results:

Composition	*Fresh Yeast*	*Dry Yeast*
Water	68.02	
Nitrogenous matter	13.10	40.98
Fatty matter	0.90	2.80
Cellulose	1.75	5.47
Starchy material	14.10	44.10
Organic matter	0.34	1.06
Mineral matter	1.77	5.54
Miscellaneous	0.02	0.05

Jones [4] has given some attention to the nucleic acids in yeasts. He prepared the potassium salt of guanylic acid from yeast. In another investigation, two dinucleotides were obtained, one yielding guanine and cytosine and the other adenine and uracil. He also described a compound of guanosine and guanylic acid. In studying the structure of yeast nucleic acid, Jones and Read [5] hydrolyzed yeast nucleic acid to yield a dinucleotide which was shown to be adenine-uracil. The

[1] Schutzemberger. Les Fermentations. Paris, 1892.

[2] Kossel. Ueber das Nuclein der Hefe, Zeit. physiol. Chemie, 3.

[3] Buchner, Ed. Alkoholische Gärung ohne Hefezellen. Berichte der deutsch. Gesellsch. 30, 1897.

[4] Jones, W. Formation of guanylic acid from yeast nucleic acid. J. Biol. Chem. 12, 31–5. The partial enzymic hydrolysis of yeast nucleic acid. J. Biol. Chem. 17, 71–80. Simpler nucleotides from yeast nucleic acid, J. Biol. Chem. 20, 25–35 (1915).

[5] Jones, W., and Read, B. E. Adenin-uracil dinucleotide and the structure of yeast nucleic acid. J. Biol. Chem. 29, 111–22. The mode of nucleotide linkage in yeast nucleic acid. J. Biol. Chem. 29, 123–6. Uracil-cytosine dinucleotide. J. Biol. Chem. 31, 39–45.

formation of such a salt cannot be explained if the two mononucleotides are joined through the PO_4. Therefore, the nucleotides in this dinucleotide, and those in nucleic acid, must be joined through the carbohydrate. This was also verified when using the method described by Jones and Gehrmann; Levene [1] takes exception to these conclusions. He calls attention to the fact that nucleotides, forming tetrabrucine salts, might be linked through the carbohydrate group of one and through the base of the other. He emphasizes that further work is necessary before deciding between the two possibilities, and that work should also be carried out on other nucleic acids such as thymus nucleic acid. Levene [2] in continuing this work secured evidence that there is no experimental proof that the nucleotides in yeast nucleic acid were bound together through the carbohydrate group. He does not believe that a tetraribose is the nucleus of yeast nucleic acid. He considers that the work in his laboratory, and in Jones', indicates the tetranucleotide structure of nucleic acid. The following three nucleotides were isolated in pure form: guanylic acid, uredinephosphoric acid and adenophosphoric acid.

Levene [3] in later investigations has written the structure of yeast nucleic acid as follows:

$$\begin{matrix} HO\diagdown \\ \quad O=P-C_5H_8O_4.C_5H_4N_5O \\ HO\diagup \end{matrix}$$

$$\begin{matrix} HO\diagdown \\ \quad O=P-C_5H_8O_4.C_4H_4N_3O \\ HO\diagup \end{matrix}$$

$$\begin{matrix} HO\diagdown \\ \quad O=P-C_5H_8O_4.C_4H_3N_3O_2 \\ HO\diagup \end{matrix}$$

$$\begin{matrix} HO\diagdown \\ \quad O=P-C_5H_8O_4.C_5H_4N_5 \\ HO\diagup \end{matrix}$$

[1] Levene, P. A. The structure of yeast nucleic acid. J. Biol. Chem. 31, 591–8, 1917.

[2] Levene, P. The structure of yeast nucleic acid. II. Uridinephosphoric acid. J. Biol. Chem. 33, 229–234.

[3] Levene, P. A. The structure of yeast nucleic acid. Jour. Biol. Chem. 31 (1917), 591–598; The structure of yeast nucleic acid. II, Uridinephosphoric acid. Jour. Biol. Chem. 33 (1918), 229–234; III, Ammonia hydrolysis. J. Biol. Chem. 33 (1918), 425–428.

General Considerations of Enzymes in Yeasts

The study of enzymes has been much advanced by Buchner who developed a procedure which allowed the extraction of the juice. Before this method was known, it was difficult to extract these enzymes which, as is generally known, are not always susceptible to passage through a membrane. They may remain on the interior of the cell and act there. Buchner's method, which consists in searching the yeast juice for the enzymes, is the only one which offers any guarantee of success.

Preparation of Yeast Juice

It is, then, by the preparation of yeast juice that we must take up the study of the enzymes. In 1897, Buchner was able to isolate the enzyme which produced the alcoholic fementation by decomposing sugar into carbonic acid and alcohol. This he called *zymase* or *alcoholase*. He did this according to the following procedure. He ground up 1000 grams of yeast after careful washing and drying. This was a difficult procedure on account of the elasticity of the yeast cells, but in order to accomplish this, it was necessary to mix the cells with fine sand and rotten stone. With the aid of a heavy iron pestle, he triturated the yeast, previously dehydrated, with 1000 grams of quartz sand and 250 grams of rotten stone in the form of a thick paste. This was expressed in a hydraulic press under a pressure of 300 to 500 atmospheres. From 400 to 500 c.c of the yeast juice were thus obtained.

The extract thus obtained is a brownish liquid with somewhat the odor of fresh yeast little or not at all dialyzable. Heating to 40–50° causes a precipitation of albumin and the liquid loses its fermenting power. It contains, along with a certain quantity of albumin (4.15 per cent), the products of tryptic digestion (albumoses, peptones, tyrosine), lecithin, a phosphorus compound (nucleic acid), 2 per cent of ash and the products of fermentation (0.53 per cent of alcohol, 0.07 per cent carbon dioxide, 0.096 per cent of glycerol, and 0.016 per cent of succinic acid).

Along with the albuminoids precipitable by alcohol and coagulable by heat, are various enzymes which we shall take up further on (endotryptase, maltase, invertase, glycogenase, lipase, etc.), and especially zymase; but this has not been isolated in the pure state. When placed with fermentable sugars (saccharose, maltose, glucose, levulose) these sugars, after a few minutes, are changed to alcohol. Further on, we shall discuss the properties of this enzyme.

ENZYMES OF PROTEIN SUBSTANCES

Proteases

Geret and Hahn have shown that yeast juice dissolves the flocculent coagulum of albuminous materials like fibrin or coagulated albumin. The juice contains a proteolytic enzyme or endotryptase, which is capable of being isolated in comparatively pure state. This enzyme plays a very important rôle in the life of the cell. The investigations of Gromow and Gregoriew[1] have shown that this endotryptase exercises a powerful action on the juice itself and that it alters and digests it rapidly at 30–35°. This action explains the rapidity with which zymase is destroyed. This enzyme is more active in an acid than in an alkaline medium, being favored by 0.2 per cent of hydrochloric acid. It seems to approach trypsin more closely in characteristics than pepsin, for Geret and Hahn have obtained a fairly complete degradation of albuminoid substance as with trypsin. The presence of leucine and tyrosine has been shown.

Endotryptase liquefies gelatin according to Hahn[2] and Hjort. This is an intracellular enzyme and not able to pass through the cell membrane. This fact makes it difficult to understand how the yeast is able to liquefy gelatin. It is common knowledge that the yeasts liquefy and peptonize, as has been shown by the works of Linder, Boullinger, Beijerinck and Astari. Also one is obliged to admit with Will that endotryptase, normally intracellular, is able under certain conditions to diffuse through a membrane. This diffusion occurs at various phases of its development, and especially in cells which are not in normal condition (dead or diseased cells).

We have stated that, according to Boullinger, certain yeasts secrete a *casease*. The formation of a curd in milk after a few months was determined. This coagulum dissolves little by little and the liquid becomes yellowish. The transformation of the casein yields tyrosine, leucine and other ammoniacal bodies. Bochicchio has verified the secretion of rennin by *Lactomyces inflans caseigrana*. Rapp has observed the presence of casease in certain yeasts. Dombrowski has shown that many of the yeasts peptonize milk strongly, especially *S. lactis v.*

According to Boullinger, there is a relation between the yeasts

[1] Gromow, T., and Gregoriew, O. Die Arbeit der Zymase und der Endotryptase in den abgetöteten Hefezellen unter verschiedenen Verhaltnissen. Zeit. f. physiol. Chemie, 2, 1904.

[2] Hahn, M., and Geret, Z. Ueber das Hefe Endotryptase, Zeit. Biol. 22, 1900.

which liquefy gelatin and those which attack casein.[1] Those which destroy the most casein liquefy gelatin most rapidly. Also it seems very probable that the liquefaction of gelatin and the peptonization of casein are due to the action of endotryptase.

According to Bokorny and Vines, yeasts contain another protease which acts like pepsin; but the existence of this enzyme is rather obscure.

Buchner and his collaborators have isolated from yeast juice an enzyme which protects albuminous matter from the action of endotryptase which they have named *antiprotease.* This enzyme seems to play an important rôle in the life of the yeast; it governs the digestive functions and balances the action of the proteases.

Rennin: The investigations of Boullinger[2] have indicated the presence of rennin in yeasts. This author has verified the existence of rennin in certain yeasts by inoculating skimmed milk and after a few months a coagulum formed which gave evidence of the presence of rennin. The curd eventually dissociated under the influence of casease.

Bochicchio has stated that the *Lactomyces inflans caseigrana* precipitated milk. The same observation has been reported by Dombrowski for other milk yeasts. Other yeasts in milk do not cause this change (yeasts of Adametz, Duclaux and Kayser). On the other hand, many other species of yeasts (*S. glutinia* of Sartory) coagulate casein without digesting it, thus secreting rennin but not casease. (Valagussa and Mafera.)

Nucleases or Enzymes of Nucleo-proteins

It seems also that yeasts contain enzymes capable of decomposing nucleo-proteins and nucleic bases. Schutzemberger has shown that xanthine, hypoxanthine and guanine are found among the autolytic products of yeasts. Recently Shiga,[3] in making yeast juice act on a solution of guanine, has detected a decrease in the quantity of this base and an increase in the xanthine which would tend to prove the presence of a guanase. According to the same author, yeast juice may also contain arginase. When submitting a solution of arginine to the action of yeast juice in the presence of toluene, Shiga has observed a disap-

[1] Diehl (Jour. Inf. Dis. 24 (1919), 347–361) has reported a type of specificity among the bacterial proteases. This author was able to detect a specificity for proteins with certain amino acids, after the bacterium had been grown on media containing these amino acids as the only source of organic nitrogen.

[2] Boullinger, E. Action de la levure de bière sur le lait. Ann. Inst. Pasteur, 2, 1897.

[3] Shiga, K. Ueber einige emsige Hafefermente, Zeit. physiol. Chemie, 42, 1904.

pearance of arginine and at the same time the formation of ornithine or urea. But guanine is not decomposed by the arginase of the yeasts.

Straughn and Jones [1] have found guanase by centrifuging aqueous extracts of yeasts made according to the following: 300 grams of yeast were macerated for 15 hours in a liter of water to which 6 c.c of chloroform had been added. The liquid thus obtained could transform guanine into xanthine and therefore contain guanase; it could not change adenine into hypoxanthine nor hypoxanthine into xanthine. Consequently it did not contain adenase nor xanthooxidase

According to H. Pringsheim [2] there exists in yeasts a special deamidase, which permits them to take nitrogen from amino acids without the production of ammonia indicating a preliminary decomposition. Finally, Effront has recently discovered in top beer yeasts an *amidase* which acts also on amino acids but produces ammonia and volatile acids.

Lipase

The existence of a lipase in the cell sap which transforms fats into fatty acids and glycerol, has been shown. This lipase seems to exert an injurious action on the zymase. This seems to be composed of a proteolytic enzyme, properly speaking, and of a coferment. Lipase decomposes the coferment.

Lipase seems to be intracellular and acts on the fats which it encounters within the protoplasm of the yeast, especially during sporulation, which are the reserve products for the cell during maturation of the ascospores.

In certains yeasts, however, lipase is able to diffuse through membranes, for van Tieghem has discovered, some time ago, *S. olei* which lives in oil and decomposes it. More recently Piedallu [3] has found a yeast which lives in oil and offers the same properties. Rogers and Jensen [4] have mentioned very many *Torula* which decompose butter.

Carbohydrate Enzymes

The investigations of Fischer and Thierfelder [5] have shown that only the sugars with carbon atoms in multiples of three are ferment-

[1] Straughn, N., and Jones, W. The nuclein ferments of yeasts. Jour. Biol. Chem. 6, 1909.

[2] Pringsheim, H. Ueber Pilzdesamidase. Biochem. Zeitschr. 12, 1908.

[3] Piedallu, A. Sur une levure qui agit sur les corps gras. Comp. Rend. Soc. de Biol. 65, 1908.

[4] Jensen, O. Bakteriologische Studien über dänische Butter. Cent. Bakt. 29, 1911.

[5] Fischer, E., and Thierfelder, H. Verhalten der verschiedenen Zucker gegen reine Hefen. Berichte d. deutsch. Gesellschaft, 27, 1894.

able. These are the glyceroses ($C_3H_6O_3$), tetroses ($C_4H_8O_4$), hexoses ($C_6H_{12}O_6$), nonoses ($C_9H_{18}O_9$), the sugars of C_{12} and C_{18} which are bisaccharides and trisaccharides and finally the polysaccharides (starch, inulin, glycogen, etc.). It is known that the bisaccharides and trisaccharides must be changed to hexoses in order to be fermentable. Fermentation, then, consists in a molecular splitting, in the course of which large molecules of sugar are changed into molecules which are much simpler, the bi- or trisaccharides into hexoses, alcohol and carbonic acid.

Polysaccharides: *Glycogenase, Amylase, Inulase:* According to the results of Wroblewsky, Cremer, Kohl and Hosaceus, and Geret and Hahn yeast juice contains a hydrolytic enzyme for glycogen, *glycogenase.* Yeasts do not act upon glycogen when it is given them as food because the glycogenase is an intracellular enzyme, and glycogen is not able to pass through the cell membrane. This glycogenase is able to act only upon the glycogen which is made by the yeast itself on the interior of the cell.

Starch, in order to be fermented, must be transformed into dextrine and maltose; then the dextrine itself is changed into maltose. This is transformed by maltose into glucose [1] which is then fermented. The exact mechanism of saccharification is not known. However, according to the investigations of Maquenne and Roux starch is composed of from 90 to 92 per cent of amylose and from 8 to 10 per cent of amylopectin. The change of starch into maltose seems to demand the action of three enzymes, amylase, amylopectinase and dextrinase. The amylase changes amylose into maltose, the amylopectinase changes amylopectines into dextrines and dextrinase changes dextrine to fermentable maltose.

Some yeasts are able to ferment starch, as *S. exiguus thermantitonum, acetethylicus, Sch. Pombe, mellacei, octosporus,* the yeast of Logos and some yeasts of Saaz and *Mycoderma sphaeromyces.*

Inulin differs from starch by its composition. Certain yeasts are able to saccharify it and ferment it on account of an *inulase* which produces levulose and not maltose, as from starch. This enzyme has been encountereed in *Sch. Pombe* and *mellacei, S. marxianus* and *thermantitonum,* certain species of the type of Saaz, and the yeasts E and F of Rose.

Trisaccharides: *Raffinase, Melibiase* and *Melizitase:* Raffinose or melitriose ($C_{18}H_{32}O_{16}$) is capable of decomposition by certain yeasts.

$$\underset{\text{Raffinose}}{C_{18}H_{32}O_{16}} + H_2O = \underset{\text{Levulose}}{C_6H_{12}O_6} + \underset{\text{Melibiose}}{C_{12}H_{22}O_{11}}$$

[1] By glucose we shall mean d-glucose or dextrose.

But some decompose it simply into levulose and melibiose, causing only the levulose to ferment; others are able to take it further. They act on the melibiose which they change to dextrose and galactose; this is fermented to d-glucose. The dissociation of raffinose is, then, the work of two enzymes, raffinase and melitriase, which split the raffinose into levulose and melibiose, and a melibiase which splits the melibiose into dextrose and galactose.

$$\underset{\text{Melibiose}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Dextrose}}{C_6H_{12}O_6} + \underset{\text{Galactose}}{C_6H_{12}O_6}$$

Saccharomycodes Ludwigii, S. marxianus, exiguus, thermantitonum, cartilaginosus, Sch. Pombe, mellacei, octosporus, the yeasts E and F of Rose, and the yeast of Logos cause raffinose and levulose to ferment but not melibiose. They contain only a raffinase. On the contrary, bottom yeast of the Frohberg and Saaz types cause melibiose and levulose to ferment while the top yeasts of these types are able to ferment only levulose and do not possess a melibiase.

According to Kalanthar, a melizitase exists in some yeasts which decomposes melizitose into dextrose and turanose.

$$\underset{\text{Melizitose}}{C_{18}H_{32}O_{16}} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Turanose}}{C_{12}H_{22}O_{11}}$$

Disaccharides: *Sucrase, Maltase, Lactase, Trehalase:* The disaccharides possess the general formula $C_{12}H_{22}O_{11}$. Four of them, saccharose, maltose, lactose and trehalose, are well known.

Saccharose is changed by sucrase or invertase to glucose and levulose. The phenomenon may be expressed by the following equation:

$$\underset{\text{Saccharose}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Levulose}}{C_6H_{12}O_6}$$

It was in the yeasts that Berthelot found sucrase for the first time. It is rather widely distributed among them. In certain species, this enzyme remains inside of the cell and only that sucrose which passes into the cell, is decomposed. The glucose and levulose thus formed diffuse through the membrane into the medium. But in *Monilia candida* and in the yeast of "Soja" not only the sucrase remains in the cell but also the glucose and levulose, whether it is not able to diffuse or whether it is destroyed as soon as it is formed. Thus it is impossible to observe the inversion of sucrose by an analysis of the fermentation mixture. But in many yeasts, sucrase is diffusible, and is able to be secreted outside of the cell. Finally certain yeasts, as *Sch. octosporus, S. apiculatus, Behrensianus, Rouxii, mali Duclauxi, P. membranæfaciens, W. belgica,* do not possess sucrase and are, consequently, unable to ferment sucrose.

Maltose in order to be assimilated and fermented must also be decomposed by an enzyme into two molecules of glucose. This is accomplished by maltase.

$$\underset{\text{Maltose}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Glucose}}{C_6H_{12}O_6}$$

Many yeasts at once decompose maltose and saccharose (*S. cerevisiae Pastorianus, intermedius, validus, ellipsoideus,* and *turbidans*). On the contrary, *S. marxianus, exiguus, Jorgensenii, Saccharomycodes Ludwigii* and *Saccharomyces guttulatus,* are able to ferment saccharose but do not ferment maltose. Maltase is then a different enzyme from sucrase. Other yeasts such as *S. apiculatus* ferment neither maltose nor saccharose, and thus possess neither maltase nor sucrase. Maltase is a reversible enzyme and transforms maltose into isomaltose.

For lactose to undergo alcoholic fermentation, it must first be changed by lactase into glucose and galactose.

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6$$

Yeasts possessing a lactase are not common. Only a small number are known. Lactase has been found in *S. Kephir* (Beijerinck), *tyrocola fragilis, acidi lactici, lactis* α and β (Dombrowski), *Zyg. lactis,* the yeasts of Duclaux, Adametz, and Kayser, and various Torula and Mycoderma isolated by Dombrowski, etc. Hunter [1] has mentioned a yeast which was able to ferment the lactose in cream. This yeast apparently possessed a lactase. Hunter also reviews the literature on yeasts which possess this enzyme. Several such instances are mentioned.

Trehalose is decomposed by trehalase into glucose and levulose. Many yeasts seem to possess a trehalase and are thus able to hydrolyze trehalose.

Kalanthar has found it in many beer and wine yeasts. *S. thermantitonum* and the bottom yeast of Frohberg (Linder) also contain trehalase.

Neuberg and Karczag [2] found that pyroracemic and oxymalic acids were fermented with the formation of carbon dioxide. Acetaldehyde was identified as the other product. This would indicate that a carboxylase removed the CO_2 from the pyroracemic acid. Carbon dioxide was also split from the following acids: acetone dicarboxylic, chelidonic, dihydroxytartaric, phenylglyoxylic and acetylenedicar-

[1] Hunter, O. W. A Lactose Fermenting Yeast Producing Foamy Cream. Journal of Bacteriology, 3 (1918) 293–300.

[2] Neuberg, C. and Karczag, L. Carboxylase, a new enzyme of yeast. Biochem. Zeit. 36, 68–75, 76–81; Chem. Abstracts, 6 (1912), 380.

boxylic acid. Neuberg and Czapski [1] demonstrated the presence of carboxylase in the juice of top yeast. Bau [2] studied the action of yeast on pyroracemic acid in the presence of certain inorganic salts. No carboxylase could be found, which confirmed Neuberg's theory that this enzyme does not diffuse from living yeast into the surrounding medium. Later Bau [3] stated that carboxylase could be demonstrated in dried yeast 20 years old. Other enzymes such as invertase, maltase, melibiase, emulsin, amygdalase, lipase and oxidase were found.

Glucosides: *Emulsin:* Fischer and Thierfelder have shown that some yeasts are able to split the α-methylglucosides (substances obtained from a condensation of methyl alcohol with glucose) into methyl alcohol and glucose. They are not able to split the β-methylglucosides, however.

According to Fischer, this action is not brought about by a special enzyme, but by maltase which possesses the ability of splitting both the α-methylglucosides and maltose. On the contrary, the β-methylglucosides are not decomposed except by an emulsin which acts to break up this substance. The investigations of Bresson [4] seem to prove, on the contrary, the existence of a special enzyme in the yeasts of Frohberg, which is sharply set apart from sucrase and maltase, and α-methylglucase.

Whatever is the truth, many yeasts are known which are able to ferment the α-methylglucosides. Some of these are *Sch. octosporus*, *Pombe*, *mellacei*, *S. thermantitonum*, the yeast of Logos, the yeast of Frohberg, and certain yeasts of Saaz.

The investigations of Henry and Auld [5] have indicated that when yeast acts on amygdaline in the presence of toluene at 40°, after 5 days about 33 per cent of the glucoside is decomposed and after 11 days, 67 to 70 per cent. From this, these authors are led to believe that an emulsin exists in yeasts. Now it seems that certain yeasts may act upon the β-methylglucosides which, according to Fischer, are not decomposed by emulsin.

[1] Neuberg, C. and Czapski, L. Carboxylase in the juice of top yeast. Biochem. Zeit. 67, 9–11, 1914. Chem. Abstracts, 9 (1915), 472.

[2] Bau, A. Carboxylase. Wochenschr. Brau. 32, 405–6. Chem. Abstracts, 11 (1916), 797.

[3] Bau, A. Yeast carboxylase; its permanence in a dry state as compared with the other enzymes of yeast. Biochem. Zeit. 73, 340–368.

[4] Bresson. Sur l'existence d'une methylglucocase specific dans la levure de bière. Comp. Rend. Acad. Sci. 151, 1910.

[5] Henry, A., and Auld, M. On the probable existence of emulsin in yeast. Proc. Roy. Soc. 76, 1905.

Bau[1] has shown that amygdalase and emulsin are present in Frohberg yeast. Experiments with Saaz yeast indicated amygdalase, but no emulsin. *S. Ludwigii*, a yeast with no maltase, acted toward amygdalin as did Frohberg yeast. This seems to indicate that the disaccharide complex of amygdalin is not identical with maltose though it contains two dextrose residues. Bokorny[2] determined the presence of amygdalin in brewers' yeast by the odor of oil of bitter almonds in incubating a mixture of yeast and amygdalin. The existence of a yeast myrosinase was also indicated by yeast and myrosin. The glucosides, arbutin, ciniferin, and salicin, were not changed by the yeast. Färber[3] stated that amygdalase, prunase and oxynitrilase, the three enzymes necessary for the complete hydrolysis of amygdalin, could be separated from bottom yeast.

Oxidizing and Reducing Enzymes

Catalases are enzymes which decompose hydrogen peroxide with the formation of inactive molecular oxygen. They seem to play a

$$2\,H_2O_2 = H_2O + O_2$$

rôle in regulating the production of hydrogen peroxide and preventing an accumulation of it. Buchner was the first to detect catalase in yeast juice.

The investigations of Tolomei, Issajew,[4] Löw[5] Henneberg, Neumann and Wender, have confirmed the existence of this enzyme. According to Neumann and Wender, two catalases exist in yeast, an α-catalase insoluble (?) in water and a β-catalase soluble in water. These enzymes, which are found in yeast juice and in yeasts killed by antiseptics, decomposed hydrogen peroxide with the formation of free oxygen.

Another enzyme similar to catalase, *philothion*, has been pointed out by Rey-Pailhade. It decolorized methylene blue and indigo carmin and transformed the sulfur in hydrogen sulfide, and the iodin in hydriotic acid. Grüss[6] has observed this same enzyme and called it hydrogenase.

[1] Bau, A. Behavior of amygdalin towards fermentation organisms. Wochenschr. Brau. 34, 29–31 (1917); Chem. Absts. 12, 403 (1918).

[2] Bokorny, T. Emulsin and myosin in the compressed yeast from Munich brewery, partly also in baker's yeast. Biochem. Zeit. 75, 376–416.

[3] Färber, E. Occurrence of emulsin-like enzymes separable from yeast cells in bottom yeast; also, the absence of myrosine in Berlin top and bottom yeast. Biochem. Zeit. 78, 264–72. Chem. Absts. 11, 1658 (1917).

[4] Issajew, W. Ueber Hefen Katalase. Zeit. physiol. Chemie, 44, 1905.

[5] Löw, O. Zur Unterscheidung zwei Arten Katalase. Cent. Bakt. 10, 1903.

[6] Grüss, J. Ueber Oxydaseerscheinungen der Hefe. Woch. für Brauerei, 17, 1903.

Hydrogenase seems to be rather widespread among the yeasts. One hundred and forty years ago, Nessler stated that if flowers of sulfur were added to a liquid undergoing alcoholic fermentation, hydrogen sulfid would be formed. We shall see further on that according to Grüss, hydrogenase plays a rôle in alcoholic fermentations.

Reductases for other sulfur compounds have been studied by various investigators. Beijerinck[1] and Kossowicz and Loew[2] were unable to find any reduction of sulfates with different strains of yeasts. Among the strains which were used by these investigators, were *Saccharomyces cerevisiae* and *Saccharomyces ellipsoideus*. Tanner,[3] however, demonstrated sulfate reduction with 9 out of 30 pure cultures of yeasts. The fungi, used by Tanner, could also split hydrogen sulfur from other sulfur compounds. Most of the cultures could attack the sulfur in sodium thiosulfate and a few reduced the sulfur in sodium sulfite. Free sulfur was also changed to hydrogen sulfide.

Oxydases are enzymes which oxidize and yield peroxides. Grüss has pointed out the presence in yeast of an oxidase which does not act on guaiac but gives a violet reaction with tetramethylphenylendiamine. This enzyme oxidized aldehydes to acetic acid and reduced fuchsin and methylene blue.

It is undoubtedly due to this enzyme that certain yeasts are able to oxidize alcohol in contact with air. Grüss believes that they play a large rôle in respiration.

Toxins

Haydruck was the first to point out the existence in yeasts of an endotoxin capable of killing them when it is extracted from the cells and introduced into the culture media. Fernbach[4] and Vulquin[5] have confirmed the existence of this toxin which seems to play toward the yeast the rôle of an antiseptic. These authors have prepared this substance in the following manner: Compressed yeast, previously dried at 70°, is macerated in a 1 per cent solution of hydrochloric acid for about 20 hours at 35–37°. The filtered macerated mixture is evaporated under reduced pressure, having been slightly alka-

[1] Beijerinck, M. W. Cent. Bakt. Parasitenk. Abt. II, 6, 194–206, 1900.

[2] Kossowicz and Loew. Gärungsphysiol. 2, 87–103 (1912).

[3] Tanner, F. W. Studies on the Bacterial Metabolism of Sulfur. II. Formation of hydrogen sulfid from certain sulfur compounds by yeast-like fungi. Jour. Amer. Chem. Soc. 60 (1918), 663–9.

[4] Fernbach, A. Sur un poison élaboré par la levure. Comp. Rend. Acad. Sci. 144, 1909.

[5] Fernbach, A., and Vulquin, E. Quelques observations nouvelles sur le pouvoir bactéricide des macérations de levures. Comp. Rend. Acad. Sci. 67, 1909.

linized with sodium hydroxids. The distillate is received in dilute sulfuric acid, and there results a liquid which, after neutralization, possesses toxic properties for the cells when introduced into them. Experiments with *B. coli* and *Staphylococcus pyogenes aureus* indicate that the yeast toxin is also poisonous to them. Like other toxins, it passes through porcelain filters and is destroyed at 100°. It is also volatile. From some of its characteristics, it seems that this toxin ought to belong to the amines. Fernbach [1] continued his study of toxic substances in yeasts by drying yeast cells at 37° C. and extracting them with dilute hydrochloric acid. The filtered extract was toxic to yeasts and bacteria. The toxic substance was destroyed at 10° C. and was volatile. Haydruck [2] has been unable to confirm these results of Fernbach.[3]

NUTRITION OF YEASTS

1. Mineral Elements

Mayer [4] has studied the mineral elements which are necessary for the yeasts. This author attempted to determine new media by the introduction of new elements. The mineral mixture which yields the bests results, is as follows:

0.1	gram	Monobasic potassium phosphate
0.1	"	Magnesium sulfate
0.1	"	Tribasic calcium phosphate
100.00	c.c	Distilled water
15.00	grams	Candied sugar.

According to these investigations, potassium phosphate plays an important rôle, after which is magnesium. It is interesting to note that these mineral elements are the same, and almost in the same proportion, as those which have been found by analysis of yeasts. The synthetic method has then confirmed the results of the analytic method.

The investigations of Elion [5] and Stern [6] have confirmed the results of Mayer and shown that the phosphates, magnesium, potassium and sulfur are indispensable elements in the life of yeasts.

[1] Fernbach, A. The poison elaborated from yeast. Comp. Rend. Acad. Sci. 149, 437–439.

[2] Haydruck, F. Yeast poison in yeast. Wochenschr. Brau. 26, 677.

[3] Fernbach, A. Toxic substance elaborated by yeast. Ann. Brasserie distillerie, 12, 361. Chem. Absts. 4 (1910), 948.

[4] Mayer, A. Lehrbuch der Gärungschemie, 1902, 5th edition.

[5] Elion, H. Studien über Hefe. Cent. Bakt. 14, 1893.

[6] Stern. Nutrition de la levure. Jour. Chem. Society, 1899 and 1901.

2. Nitrogenous Substances

The nitrogenous substances may be divided into four groups: ammonia, nitrates, albumins, and their derivatives, such as amides and amines. Since the investigations of Boussingault, it has been known that the nitrates play an important rôle in the nutrition of higher plants. The investigations of Muntz have shown that ammonia is also assimilated by higher plants, but it is only a substance of medium importance. In the nutrition of yeasts, ammonia salts (phosphates and sulfates), on the contrary, play an important rôle while the nitrates are generally not assimilated.

Pasteur was the first to establish that the ammonium salts were good foods for the yeasts. The later investigations by Duclaux and Laborde, and Laurent have confirmed these results. The experiments of Laurent have indicated that yeasts do not assimilate nitrates. According to this author, they would have to reduce the nitrates to nitrites, substances which are toxic. Beijerinck, however, has stated that certain yeasts, such as *S. acetethylicus*, are able to assimilate nitrates. Since then, the investigations of Kayser,[1] and Fernbach and Lanzenberg[2] have shown that, if nitrates are injurious to multiplication, they have a favoring influence on the zymase in fermentation.

The relation of albuminoid substances to the metabolism of yeasts is very obscure. According to Pasteur and Ad. Mayer, the yeasts are unable to use egg white or blood fibrin. These substances do not pass through the cell membrane, and the endotryptase of the yeasts is an intracellular enzyme which does not pass easily to the outside of the cell. We have seen that, according to Boullinger, certain yeasts inoculated into milk develop very slowly and produce, after a few months, a curd which slowly liquefies with the formation of ammonium salts, tyrosine, and leucine. There is then a dissolution and digestion of the casein by the yeast. It is known, on the other hand, that certain species of yeasts liquefy gelatin. It must be admitted that, under special conditions, endotryptase may pass through the cell membrane.

On the contrary, if yeasts do not accommodate themselves to these compounds, they easily assimilate the dialyzable derivatives of them, such as the albumoses and peptones. It is curious to note that

[1] Kayser, E. Influence des nitrates sur les ferments alcooliques. Comp. Rend. Acad. Sci. 150, 1910.

[2] Fernbach, A., and Lanzenberg, A. De l'Action des nitrates dans la fermentation alcoolique. Comp. Rend. Acad. Sci. 151, 1910.

they are able to utilize also as sources of nitrogen, certain enzymes such as pepsin (Mayer and Heinzelmann).

According to more recent investigations, the derivatives of albuminoids (amides, amino acids, and leucomaines) are assimilated more easily than the albumoses and make up the desirable nitrogenous substances for the yeasts. The work of Rettger has shown the same thing for the bacteria.

Waterman[1] stated that the amino group is an especially suitable source of nitrogen. This depends on the presence of one or more acid amide groups which are not available for nutrition. Waterman points out that this selective action of yeasts may be used to separate closely related compounds. Asparagin and aspartic acid are utilized while succinamic and succinamide, which contain only the acid amide groups, are not assimilated. Cinnamamide is not assimilated while α-aminocinnamamide is used.

Neubauer and Fromherz[2] fermented *dl* phenylaminoacetic acid ($C_6H_4OH(NH_2)COOH$) in the presence of 10 per cent of cane sugar for three days. There was left an amount of undecomposed acid which was usually more or less *l*. By means of certain methods phenylglyoxylic acid hydrazone was obtained. Sodium succinate, benzyl chloride, *p*-hydroxyphenyl ethyl alcohol (perhaps from yeast tyrosine), *l*-acetylphenylamino acetic acid were obtained. Para-hydroxyphenylpyruvic acid was also fermented. These authors are led to construct the path from amino acid to the next lower alcohol as follows:

$$RCH(NH_2)COOH = RC(OH)(NH_2)COOH$$
$$= RCOCOOH = RCHO = RCH_2OH.$$

The processes thus involved are oxidation, decarboxylation, acid reduction. The alcohol acid RCHOHCOOH and the acetylamino acid result from secondary reactions.

Kossowicz[3] found that yeasts could utilize nitrates. Bokorny[4] found that nitrates were unaltered and not assimilated. The simple amines, such as ethyl amine, were also unfavorable. The presence of sugars was found to be necessary to keep down the bacteria. With various sources of nitrogen in the medium, the following increases were observed in dried yeast:

[1] Waterman, H. J. Nitrogen nutrition of compressed yeast. Zent. Biochem. Biophys. 16, 276.

[2] Neubauer, O., and Fromherz, K. The decomposition of amino acids in yeast fermentation. Zeit. physiol. Chem. 70, 326–350.

[3] Kossowicz, A. Behavior of yeasts and molds towards nitrates. Biochem. Z. 67, 400–19, 1914.

[4] Bokorny, Th. Sources of nitrogen of yeasts. Chem. Ztg. 40 (1916), 366–368.

$(NH_4)_2$ + sucrose	=	71.8	per	cent	increase
" + dextrose	=	113.0	"	"	"
Asparagin + sucrose	=	103.7	"	"	"
Aspartic acid + "	=	61.3	"	"	"
Leucine + "	=	90.3	"	"	"
Tyrosine + "	=	61.3	"	"	"
Glycine + "	=	25.8	"	"	"

With *somatose* (flesh albumose) there was a decrease of 9.7 per cent which would seem to indicate that the albumoses must be further decomposed. Peptone with sucrose gave an increase of 177 per cent while peptone alone gave 152 per cent.

Lindner and Wüst [1] find that urea can serve yeasts and molds as sources of nitrogen but not for carbon. Bokorny [2] has measured the increase in development of yeast when nitrogen is taken from urea, by the dry weight. Considerable growth took place when the yeast was grown in urine to which sugar had been added. The urea and not the hippuric acid is said to be the source of the nitrogen.

Hoffman [3] has shown that the addition of ammonium chloride to bread dough saved 30 per cent of the yeast ordinarily used. Experiments were carried out which showed that this nitrogen went to construct yeast protein. Good arguments are presented which show that this salt does not go for other purposes. Ehrlich [4] stated that ammonium salts were readily changed into yeast protein. Delbrück and Classen [5] have used ammonium salts for cultivating yeast. Völtz [6] found that the composition of yeast grown on mineral salts was like that grown with other sources of nitrogen.

Kossowicz and Gröller [7] have stated that the thiocyanates will serve yeasts as source of nitrogen and sulfur but not carbon.

[1] Lindner, P., and Wüst, G. Assimilation of urea by yeasts and molds. Wochenschr. Brau. 30, 477–9. Chem. Absts. 8 (1914), 727.

[2] Bokorny, Th. The increase in dry weight of yeast when urea is used as the source of nitrogen. Biochem. Zeit. 82 (1917), 359–390; The culture of yeast in the presence of air with the use of urea as source of nitrogen and with different sources of carbon. The quotient of sugar assimilation. Biochem. Z. 83 (1917), 133–164. Chem. Absts. 12 (1918), 1203.

[3] Hoffman, C. H. The utilization of ammonium chloride by yeast. Jour. Ind. Eng. Chem. 9 (1917) 148–151.

[4] Ehrlich. Biochem. Z. 18, 391–423.

[5] Delbrück. Classen. A new method for increasing the production of yeast. Z. Ver. Deut. Ing. 59 (1915), 844.

[6] Völtz. Utilization of the animal organism of yeast produced from sucrose and nutritive mineral salts. Z. Spiritusind. 38 (1915) 235–6.

[7] Kossowicz, A. and Gröller, L. Thiocyanates as a source of carbon, nitrogen and sulfur for molds, yeasts and bacteria. Zeit. Gärungsphysiologie, 2, 59–65. Chem. Absts. 7 (1913), 808.

The investigations of Mayer, Haydruck and Kusserow, Schulz, Thomas [1] and Lindner, have shown that, among the amides, allantoin, asparagin, and urea are assimilable by yeasts. It has been pointed out, however, that the investigations of Shiga, Straughn and Jones have indicated in yeast juice the presence of a guanase which transforms guanine into xanthine, and of an arginase which will split xanthine and ornithine into urea. According to Mayer, caffein, creatin, and creatinin are not acted upon by yeasts.

The investigations of P. Lindner,[2] and his collaborators Rülke and Hoffmann, have shown that yeasts may assimilate products of their autodigestion. Among those, tyrosine, leucine, adenine, asparagine, aspartic acid and ammonium sulfate are most easily assimilated; finally, choline is used. All of the yeasts, however, do not act in the same manner in this relation. It is thus that the top and bottom yeast of the brewery and distillery types and yeast juice easily assimilate tyrosine, leucine, adenine, aspartic acid, guanidine, arginine, hypoxanthine, histidine, uracil, choline, thymine, potassium nitrate and ammonium sulfate. *Mycoderma* and the species of the genus *Willia* and *Pichia* use almost all of the products of autolysis.

More recently, Ehrlich [3] has stated that leucine and isoleucine are especially desirable compounds for yeasts which add a molecule of water, splitting them into isoamyl alcohol (or amyl) and ammonia. The ammonia is used by the yeast. Effront [4] has also given evidence of the existence of an amidase which acts on amino acids but without giving alcohols, transforming them into ammonia and volatile acids. Pringsheim [5] admits the existence of a *desamidase* which allows yeasts to take their nitrogen from amino acids but without the production of ammonia. The investigations up to the present, then, seem to indicate that the amino acids make up a better source of nitrogen for yeasts. Data from Rettger's laboratory allow similar conclusions for the bacteria. Koser and Rettger [6] have shown that the amino acids

[1] Thomas, P. Sur la nutrition azotée de la levure. Comp. Rend. Acad. Sci. 131, 1910.

[2] Lindner, P. Rülke, P., and Hoffmann. Wochenschr. Brauerei, 22, No. 40. Lindner, P., and Stockhausen. Wochenschr. Brauerei 3, No. 40 and Bioch. Centr. IV.

[3] Ehrlich, F. Uber die Spaltung racemischer Aminosäuren mittels Hefe. Biochemische Zeitschr. 8, 1908.

[4] Effront, I. Action de la levure de bière sur les acides amides. Comp. Rend. Acad. Sciences, 146, 1908.

[5] Pringsheim, H. Ueber die Stickstoffernährung der Hefe. Biochemische Zeitschrift, 3, 1907.

[6] Koser, S. A. and Rettger, L. F. Studies on Bacterial Metabolism. The Utilization of Nitrogenous Compounds of Definite Chemical Composition. Jour. Inf. Dis. 24 (1919) 301–321.

serve bacteria through several generations while, in other papers, it has been shown that the more complex split products of protein could not.

Jodin [1] and Hallier [2] attributed to yeasts the ability to fix atmospheric nitrogen. Woff and Zimmermann,[3] however, could not confirm these statements. Zikes [4] isolated a pseudo-yeast, *Torula wiesner*, to which he attributed the ability to fix atmospheric nitrogen. He isolated this organism from laurel leaves, and secured a fixation of 2.3–2.4 mg. per gram of glucose. Löhnis and Pillai [5] secured but slight fixation with a *Torula*. With *Dematium pullulans* there was a greater fixation. Lipman [6] found that yeasts and pseudo-yeasts could fix atmospheric nitrogen. The action went better in solutions containing dextrose. Lindner and Naumann [7] could secure no fixation in a solution containing 5 per cent of dextrose, .025 per cent of magnesium sulfate, 0.5 per cent of mono potassium phosphate and .025 per cent of asparagin. Such results are in sharp contrast with those of other investigators. Kossowicz [8] reaches the conclusion that while yeasts can live with but a very small amount of nitrogen, they do not have the power to fix atmospheric nitrogen. Other nitrogenous compounds may be taken from the air.

Schwarz [9] made an interesting study on the effect of adrenalin on unicellular organisms. He found that this substance acted on organisms without nerves just as it did on higher organisms. Large quantities of sugars were used, as evidenced by much CO_2. The ability to utilize non-diffusible substances was acquired (glycogen, casein, alanine). These were changed to fermentable sugars. Further experiments with glycogen, starch, alanine, and sodium aspartate, gave CO_2 when adrenalin was present, otherwise not.

Lindner [10] studied the various sources from which a yeast could

[1] Jodin, Compt. rend. Acad. Sci. 55, 612.

[2] Hallier, Zeit. für Parasitenkunde. 1, 129.

[3] Woff and Zimmermann. Jahresbericht der Ag. Chemie, 13–15, 169.

[4] Zikes, Sitzungsber. Akad. Wien. mathe. naturw. Kl. 118, 1091.

[5] Löhnis, F. and Pillai. Cent. Bakt. Abt. 2, 20, 799.

[6] Lipman, C. B. Nitrogen fixation by yeasts and other fungi. J. Biol. Chem. 10, 169–182.

[7] Lindner, P. and Naumann, C. W. Assimilation of nitrogen of air by yeasts and molds. Wochenschr. Brau. 30, 589–592.

[8] Kossowicz, A. Question of the assimilation of elementary nitrogen by yeasts and mold fungi. Biochem. Z. 64, 82–5. Fixation of elementary nitrogen by saccharomycetes (yeasts) and molds. Z. Gärungsphysiologie, 5, 26.

[9] Schwarz, O. The action of adrenalin on the monocellular organism. Wienklin. Wochenschr. 24, 267–8. Decomposition of nitrogenous substances by yeasts. Biochem. Z. 33, 30–1.

[10] Lindner, P. Results obtained in fermentation and assimilation experiments with yeasts. Chem. Ztg. 34, 1144.

take nitrogen. Compounds with long hydrocarbon chains were easily assimilated. The ring structures, such as histidine, were used with more difficulty. Leucine, adenine, and lysine were easily assimilated, but thymine, uracil, choline, hypoxanthine more difficultly. Adenine, since its nitrogen is in the side chain, was more easily assimilated than hypoxanthine. The more aerobic yeasts were found to utilize more difficultly assimilable nitrogen more easily.

Zalesky and Israelsky [1] found that the protein content of yeast remained constant in fermentation. Asparagin and glutamic acid support synthesis of protein while glycocoll and phenylalanine do not.

Thomas [2] and Kolodziejska [3] found two new proteins in yeasts. One belonged to the casein group and the other to the vegetable albumins. This latter was named cerevisin.

Meisenheimer [4] studied nitrogen substance in yeast by autolysis in presence of toluene. All of the common amino acids were found among the cleavage products of yeast protein. Glucosamine, so often looked for in vain, was demonstrated to be present. Nitrogen in yeast protein is distributed as follows:

Ammonia nitrogen	11 per cent
Alloxur bases (nuclein bases) nitrogen	7 per cent
Arginine-histidine nitrogen	22 per cent
Lysine-choline nitrogen	4 per cent
Monoamino acid nitrogen	56 per cent

Haydruck [5] has shown that yeasts are suitable foods and that they should be looked upon favorably as constituents in the human diet.

Ehrlich [6] has stated that amino acids are deaminized and the rest of the molecule is discharged as fatty acid or alcohols. Sugar is said to be the sole source of carbon. To secure data with regard to what products in sugar decomposition went to make up the complex yeast proteins, he grew *Willia anomala*, Hansen, in solutions containing only mineral salts, tyrosine and either glycerol, ethyl alcohol, methyl

[1] Zalesky, W. and Israelsky, W. Synthesis of protein in yeast. Ber. deut. Bot. Ges. 32 (1914), 472–9.

[2] Thomas, P. Protein substances of yeast. Comp. rend. Acad. Sci. 156, 2024–7.

[3] Thomas, P. and Kolodziejska, S. Protein matter of yeast and its products of hydrolysis. Comp. rend. Acad. Sci. 157, 243–6.

[4] Meisenheimer, J. The nitrogenous substance of yeast. Wochenschr. Brau. 32 (1915) 325–6.

[5] Haydruck, F. The utilization of yeasts. Brewers Journal, 48, 57–58. 1912.

[6] Ehrlich, F. The formation of the plasma in yeasts and molds. Biochem. Z. 36, 447–97; Chem. Absts. 6 (1912) 240.

alcohol, amyl alcohol or lactic acid. Cultures in glycerol and ethyl alcohol grew as well as the control in sucrose. Cultures in lactic acid, methyl alcohol and amyl alcohol grew slightly, and formed sufficient tyrosol for isolation. Since tyrosol corresponding to nearly all of the tyrosine was obtained, it shows that when the carbon diet is limited to simple compounds, the yeast does not utilize the carbon of amino acids.

3. Hydrocarbon Compounds

The yeasts, being, like the fungi, without chlorophyll, are not able to take their carbon from the atmosphere. They have to resort to other compounds as sugars, aldehydes, acids, etc.

The hydrocarbon metabolism of yeasts ought to be looked at from two standpoints. One should distinguish the hydrocarbon metabolism of the yeasts during the aerobic life, that is the plant-yeast, and also during fermentation, yeast-ferment. In the two cases, they act differently. The first of these will be treated here, and the other when we take up alcoholic fermentation.

From the experiments of Laurent,[1] it is evident that the alcohols, aldehydes, ethers, fatty acids, amides, glycocoll, hydroquinone, and cellulose are not able to liberate their carbon to the yeasts. On the contrary, the yeasts are able to take it from acetates, lactates, citrates, tartrates, malates, succinates, tartaric acid, malic acid, succinic acid, lactic acid, glycerol, from sugars of the $C_6H_{12}O_6$ and $C_{12}H_{22}O_{11}$ series, and from substances capable of transforming into glucosides dextrine lecithin, asparagin, peptones, etc. Bokorny has also reached about the same conclusion.

It seems that alcohol, which these authors regard as not used by yeasts, is able, however, to be used by certain species.[2] Thus it is that recent investigations by Trillat and Sauton,[3] Kayser and Demolon [4] indicate that they oxidize alcohol to the aldehyde.

The investigations of Lindner and Saito [5] indicate that maltose

[1] Laurent, E. Nutrition hydrocarbonée et formation du glycogène chez la levure de bière. Ann. Past. Inst. 3, 1889.

[2] We shall see that yeasts are able to live for many years in liquids which they have fermented. It is probable that they use the glycerol and succinic acid (which are regarded by Laurent as being able to supply the needs of yeasts for carbon). For certain species alcohol seems to be the source of carbon.

[3] Trillat and Sauton. L'aldéhyde acétique est-il un produit normal de la fermentation alcoolique? Ann. Inst. Past. 24. 1910.

[4] Kayser and Demolon. Sur la vie de la levure après la fermentation alcoolique. Comp. Rend. Acad. Sci. 149. 1909.

[5] Lindner, P. and Saito, K. Assimilierbarkeit verschiedener Kohlehydrate Hefe. Woch. Brauerei No. 41. 1910.

is best adapted to yeast metabolism. This sugar is assimilated by practically all of the yeasts. Dextrine is transformed by certain varieties (Mycoderma and Torula), but is not well adapted to others. Sucrose which is so easily fermented does not play any rôle in assimilation. The same is true with regard to glucose, levulose, raffinose, and arabinose. Finally, lactose is not assimilated except in isolated cases. Lindner,[1] in a later publication, stated that maltose is easily available as a nutrient sugar, glucose, fructose, and cane sugar being less valuable. In fact sucrose was often valueless.

On the other hand, the experiments of these authors have showed that there is no relation between the fermentability of a sugar and its use as an nutrient. Thus it is that one frequently meets yeasts which, in functioning aerobically, energetically assimilate a sugar, while, functioning anaerobically, they are unable to ferment it. One may encounter, although rarely, a yeast which is able to ferment a sugar and not able to use it as a nutrient. Such is the case with *S. Ludwigii, exiguus, cartilaginosus* and *Sch. Pombe* and *mellacei* which produce an active fermentation of glucose, levulose and saccharose but are unable to assimilate any of them.

Kluyver [2] attributed the statements that yeast is able to assimilate maltose to the fact that the maltose contained glucose. When the maltose was purified and freed from the glucose no assimilation was secured. Lindner [3] has shown that maltose is easily assimilated by yeasts. Glucose, sucrose, and fructose were less satisfactory.

It is known since the work of Errera that glycogen is abundant in yeast cells. Since it is there so abundantly, it seems to have considerable importance in the life of the yeasts. The study of the conditions for its formation is very interesting and may explain many facts with regard to the hydrocarbon nutrition of yeasts. This study has been made by Laurent who has stated that glycogen is able to be formed at the expense of the following substances:

Lactates
Succinic acid and ammonium succinate
Malic acid and malates
Mannite
Sugars of the $C_6H_{12}O_6$ and $C_{12}H_{22}O_{11}$ series
Glycogen

[1] Lindner, P. The results obtained in fermentation and assimilation experiments with yeasts. Chem. Ztg. 34, 1144. Chem. Absts. 6 (1912) 1050.

[2] Kluyver, A. J. Assimilability of maltose by yeasts. Biochem. Zeit. 52, 486–493.

[3] Lindner, P. The results obtained in fermentation and assimilation experiments with yeasts. Chem. Ztg. 34, 1144.

Gum arabic
Erythrodextrine and dextrine
Mucic acid
Asparagin and glutanine
Salicine, amygdalin, and other glucosides
Egg albumin
Peptones from fibrine and casein.

It is stated, according to Laurent, that glycogen is able to serve in the hydrocarbon nutrition of yeasts and the production of glycogen in the cell. This statement is without doubt in error. From the investigations of Koch and Hosaeus,[1] it has been established that glycogen is not absorbed by yeasts, for it is not diffusible through the cell membrane any more than the glycogenase enclosed in the cell.

The conclusions of Laurent have been confirmed, in most part by Cremer. This author has also stated that yeasts deprived of their glycogen by autofermentation phenomena, which we shall study further, produce it after a few hours if they are placed in a solution with sugar (saccharose, levulose, glucose, d-galactoce, d-mannose) but not if furnished with arabinose, rhamnose, sorbose, lactose, glycerol or glycogen.

According to Laurent, Boullinger, and Kayser and Meissner, glycogen is rare or completely absent at the beginning of fermentation; it increases progressively and soon reaches a maximum. It disappears at the end of fermentation. These results are absolutely confirmed by those of Wagner, Kohl and Guilliermond. It seems that toward the middle of the fermentation, glycogen accumulates in the cell much more quickly than it is consumed.

The investigations of Lindner and Will indicate that glycogen is unevenly distributed in the yeast cell and is able to exist under very variable conditions. Thus it is that Lindner has observed glycogen in yeasts which had been cultivated on gelatin for 4 months. The same is true of most reserve products. It is difficult to state accurately the conditions under which the formation of glycogen is greater than the expenditure.

These authors have come to regard glycogen as a transitory substance in the cell and intermediary between the sugars and alcohols. According to Grüss, it constitutes, as we shall show later on, an exclusive substance destined for respiration (in presence of air) and for fermentation (in the absence of air). Glycogen is formed from the sugars which are dissolved by the cell and is transformed either into

[1] Koch, A. and Hosaeus. Ueber einen neuen Froschlauch der Zuckerfabriken. Cent. Bakt. 16, 1894.

carbonic acid and water in aerobic life, or into carbonic acid and alcohol in anaerobic life.

Kohl holds the same opinion based on the following: Glycogen is especially abundant in yeast cells during active fermentation. It is not found in cells about to sporulate or in ascospores. Our observations have shown on the contrary that glycogen is very abundant, not only during fermentation but also during the formation of ascospores in the course of their maturation. Part is used in the formation of the ascospore while the rest is kept in reserve for their germination. The investigations of Will have shown that the durable cells contain large quantities of glycogen. These facts do not exclude the theory of Grüss, for it is possible that glycogen is a reserve product especially for respiration.

Henneberg [1] states that glycogen may occur in both normal and abnormal yeast. Since yeast cells containing more that 53 per cent of protein usually contain little glycogen, it is probable that yeast cells in potato mashes, etc., will contain little. Bruschi [2] found that antiseptics, such as chloroform, ether, thymol and formalin, did not completely stop the formation of glycogen even though fermentation was impeded. The production of alcohol determined the amount of glycogen formed. It is stated that glycogen is formed by the condensation of some intermediate product of fermentation. Kullberg [3] reported an inverse relation between the nitrogen content of the yeast cell and the glycogen content.

Will and Heuse [4] found that ethylacetate satisfied the carbon requirements of yeast and that they could grow without the presence of organic matter. Lindner and Cziser [5] found alcohol a source of carbon. Stockhausen [6] confirmed this opinion. Lindner [7] has re-

[1] Henneberg, W. Amount of glycogen in differently fed yeast cultures. Biedermann's Zentr. 1912. 277–8; Chem. Absts. 6, 1917–18. 1912.

[2] Bruschi, D. Formation of glycogen in the yeast cell. Att. accad. Lincei, 21, I, 54–60; Chem. Absts. 6, 1018–19, 1912; Cent. Bakt. Abt. II, 35, 316; Chem. Absts. 7 (1913) 495.

[3] Kullberg, S. Simultaneous change in the content of glycogen, nitrogen and enzyme in living yeast. Zeit. physiol. Chem. 92, 340–359. (1914); Chem. Absts. 9 (1915), 471.

[4] Will, H., and Heuse, R. Ethyl acetate as a source of carbon for yeasts and other budding fungi. Zeit. ges. Brauw. 35, 128–9, Chem. Absts. 6 (1912) 1626.

[5] Lindner, P. and Cziser. Alcohol, a more or less excellent nutrient medium for different organisms. Wochenschr. Brau. 29, 1–6; Chem. Absts. 6 (1912) 1916.

[6] Stockhausen, F. Alcohol assimilation by yeasts. Chem. Ztg. 35, 1197. Chem. Absts. 6 (1912) 3106.

[7] Lindner, P. Non-assimilation of methyl alcohol by microörganisms capable of assimilating ethyl alcohol. Z. Spiritusind. 35, 185; Chem. Absts. 6 (1912) 1917.

ported that *S. membranaefaciens* could take its carbon from ethyl but not from methyl alcohol. Bokorny [1] studied the sources of carbon for yeasts and stated that urea cannot supply carbon for yeasts. This is confirmed by Lindner and Wüst [2] who found that urea could supply nitrogen but not carbon. Pentoses were found by Bokorny to be not fermented but could serve as a source for carbon. Others have shown that different organic acids, glycerol, asparagin, peptone, etc., could be used. Will [3] found that, in mineral media, esters could act as a source of carbon. Lindner [4] studied the assimilability of the various carbohydrates. He investigated dextrose, mannose, galactose, levulose, trehalose, sucrose, maltose, lactose, melibiose, raffinose, α-methylglucoside, xylose and rhamnose. The simple sugars, trehalose, sucrose and maltose, were fermented. Lactose was not. In certain cases, there was a questionable fermentation of xylose and rhamnose. Melibiose was not fermented by top yeast. There seemed to be a marked difference in action toward α-methylglucoside. In a later paper Lindner [5] again found maltose better than other sugars.

Bokorny [6] has stated that external factors have great influence in fermentation and assimilation studies. Light is not important for the yeasts but plenty of air is important. The increase in dry substance in this experiment was taken as the criterion of assimilation. Assimilation was promoted by free KOH at certain concentrations.

Kita [7] found that purified maltose was less easily assimilated than unpurified. He attributed this to the fact that in the impure maltose there was an oryzanin-like compound. The same thing was observed by Kluyver.[8]

[1] Bokorny, T. The formation of protein from different sources of carbon. Münch. med. Wochenschr. 63, 791–2; Chem. Absts. 11 (1917) 2813.

[2] Lindner, P. and Wüst, G. Wochenschr. Brau. 30, 477–9. Chem. Absts. 8 (1914) 727.

[3] Will, H. Influence of esters on yeasts and other budding fungi. Cent. Bakt. Abt. II. 38, 539–76. Chem. Absts. 8 (1914) 357.

[4] Lindner, P. Assimilation of various carbohydrates by different yeasts and the like. Wochenschr. Brau. 47, 561–563. Chem. Absts. 6 (1912) 1808.

Lindner, P. and Saito, K. The assimilation of various carbohydrates by different yeasts. Wochenschr. Brau. 27, 509–13; Chem. Absts. II, 476, 5 (1911) 1489.

[5] Lindner, P. The results obtained in fermentation and assimilation experiments with yeasts. Chem. Ztg. 34, 1144.

[6] Bokorny, Th. Relation between sugar fermentations and sugar assimilation. Allgem. Brau-Hopfen Ztg. 57, 447–80; Chem. Absts. 12 (1918) 847.

[7] Kita, G. The question of the assimilability of maltose by yeasts. Zeit. Gärungsphysiologie, 4, 231–4. Chem. Absts. 8 (1914) 3809.

[8] Kluyver, A. J. Assimilability of maltose by yeasts. Biochem. Z. 52, 486; Chem. Absts 8 (1914) 359.

Lindner,[1] using *Saccharomyces membranaefaciens*, which assimilates ethyl alcohol in the absence of other sources of carbon, could not find an assimilation of methyl alcohol.

Stockhausen [2] inoculated a mineral nutrient solution containing $(NH_4)_2SO_4$ as the source of nitrogen and 4 per cent of alcohol as the source of carbon. Excellent yeast growth was secured in a few days. This author argues that alcohol, in this case, was a food from which plasma, cell membrane and fat could be built. The same fact was established by Lindner and Czier.[3]

Rubner [4] believes that other than physical conditions control assimilation and nourishment of yeasts since they take what sugar is needed irrespective of its concentration. Rubner found that live yeast, as well as yeast killed with toluene, quickly took up sugar from a solution without fermentation, while yeast heated to 100° C. did not. This author regards the yeasts as organisms possessing great energy transformations per unit mass. Lindner [5] found that growth took place at the expense of atmospheric nitrogen. Ethyl alcohol and free ammonia were used to build protoplasm. *Saccharomyces acetethylicus* assimilated nitrogen from nitrates. Urea provided assimilated nitrogen particularly if maltose was present. Maltose was found to be the best source of carbon. Melibiose and raffinose are readily assimilated by yeasts even by some which do not ferment them.

Respiration

We have seen that yeasts placed in contact with air, act like ordinary plants without causing alcoholic fermentation. Like all living matter, they respire; they take in oxygen and liberate carbon dioxide. The investigations of Schutzemberger, Grehant and Quinquand [6] have shown that they are very eager for oxygen.

Schutzemberger has stated that fresh yeast put into water well aerated at fermentation temperature is obliged to live at the expense

[1] Lindner, P. Non-assimilability of methyl alcohol by microörganisms capable of assimilating ethyl alcohol. Zeit. Spiritusind. 35, 185; Chemical Abstracts, 6 (1912) 1917.

[2] Stockhausen, F. Alcohol assimilation by yeasts. Chem. Ztg. 35, 1197. Chem. Absts. 9 (1912) 4106.

[3] Lindner, P. and Czier, S. Alcohol a more or less excellent nutrient medium for different organisms. Wochenschr. Brau. 29, 1–6; Chem. Absts. II, 476, 6 (1912), 1916.

[4] Rubner, M. Assimilation of nourishment by the yeast cells. Cent. Bakt. Abt. II. 38 (1914), 128. Chem. Absts. 9 (1915), 93.

[5] Lindner, P. Results of recent experiments on assimilation by yeasts and molds. Zeit. angewandte Chemie, 25, 1175. Chem. Abstracts 7 (1913) 2055.

[6] Grehant and Quinquand. Ann. des. Sc. nat. Bot. 1889.

of its reserve products, absorbing oxygen and giving off carbon dioxide. Yeasts are also able to take oxygen from compounds in which it is loosely combined. It is thus, as Schutzemberger and Risler have stated, that when fresh yeast is placed in arterial blood or in a solution of hemoglobin saturated with oxygen, the color passes from a deep red to a bluish black. In this case the yeasts take their oxygen from the blood and act like tissue cells in the animal body. While yeasts are able to take oxygen only from such unstable combinations as hemoglobin, they are not able to get it from compounds which hold it firmly. For instance, they are without action on indigo carmin which some bacteria decolorize so strongly.

The respiratory activity measured by the oxygen consumed in a unit time by a unit weight of yeast, varies with the temperature. It is very feeble at 10°, increases slowly up to 18°, and attains its maximum towards 60°. It falls quickly after the death of the yeast. The experiments of Grehant and Quinquand have given the same results. They have shown that respiration diminishes a little and reduces itself to a minimum during the anaerobic life of the yeast, but never totally disappears. As has been stated before, Grüss regarded glycogen as important in respiration. According to this author, glycogen is a reserve product utilized in respiration and fermentation. It is by means of their oxidases that yeasts oxidize the glucose secured by hydrolysis of glycogen, transforming it into carbon dioxide and water.

ALCOHOLIC FERMENTATION

General Characteristics of Alcoholic Fermentation

Conditions Necessary for Its Production

While the molds produce very quickly on the surface of liquids a vigorous vegetation, and thus live in contact with air, the greater number of the yeasts develop at the bottom of culture media in the form of a sediment, and it is only under exceptional circumstances that they develop on the surface in the form of a pellicle often called a scum.

When cultivated in a dish containing sugar solution in a thin layer, the supply of air is sufficient to allow a vigorous growth of the yeast. Under these circumstances, it decomposes the sugar, using part for maintaining protoplasm or constructing new substance, and transforming the rest by oxidation to carbon dioxide and water. In a word, it is aerobic and acts like other plants.

The activities are different when it is put into a flask almost completely filled with a sugar solution and to which air does not

have access. The yeast grows at the bottom of the flask and finds a bad supply of oxygen. In this case it uses little sugar for maintaining itself and scarcely multiplies; the rest of the sugar is changed into alcohol and carbon dioxide by the enzymes.

In anaerobic life the yeasts are not able to secure their energy by oxidation. Quite another chemical change is involved; this is the enzymatic change of sugar into alcohol and carbon dioxide. One easily conceives that much less energy is secured by this process than by an oxidation. Much of the energy will be used for building up a very small quantity of new protoplasm. The transformation of sugar into alcohol will be considerable for a minimum growth of the yeast. The memorable researches of Pasteur [1] have enriched our information with regard to this change. This illustrious savant, by a series of experiments, demonstrated that the scarcer the amount of oxygen, the greater was the amount of fermentation.

The best means of propagating a yeast under aerobic conditions is, as we have stated above, growing it in a shallow dish with a few centimeters of nutrient medium. Under such conditions, Pasteur, at the end of 24 hours, has obtained 24 milligrams of yeast cells with a consumption of 98 milligrams of sugar. No trace of alcohol is found in the medium. At the end of 48 hours, Pasteur obtained 127 milligrams of yeast cells for 1.04 grams of sugar decomposed. The yeast was almost exclusively an agent of oxidation and acted like other plants. It consumed a large part of the sugar for its maintenance and multiplication and its weight increased in a considerable proportion.

It does not act like this when put into a flask to which air does not have free access. For 10 grams of sugar decomposed Pasteur secured only 0.44 gram of yeast cells. The yeast had scarcely multiplied; on the contrary, the proportion of sugar changed to alcohol became greater.

Pasteur has continued his experiments by putting a thin layer of liquid into the same flask. This time, the fermentation was longer and the weight of the yeast less perceptible; but the same proportion of alcohol is found as in the alcohol fermentations so-called. In this last experiment, the liquid in the flask was aerated, retaining a small quantity of oxygen which the yeast utilized at the beginning of its development. On the other hand, the yeast may come from cultures which are in contact with air; the cells then have been able

[1] Pasteur. Mémoire sur la fermentation alcoolique. Ann. de Chim. et de phys. 1859. Influence de l'oxygène sur le dév. de la levure et de la ferm. alc. Bull. Soc. Chim. 1861.

to accumulate sufficient oxygen. Pasteur also repeated the experiment by eliminating these two sources of oxygen. For this he inoculated a trace of the yeast taken at the end of a fermentation and which had not been in contact with air, into a flask almost completely filled with sugar solution which had been boiled. Under such conditions, the fermentation was very slow. Pasteur made it endure three months. At the end of this time, 45 grams of sugar had disappeared and only 0.255 gram of yeast had been formed. The yeast, then, did not develop. Thus from these experiments one sees the weight of sugar which a unit weight of yeast is able to decompose into alcohol and carbonic acid and at the same time diminish the activity of life and the power of reproduction.

All of this demonstrates that fermentation is correlated with anaerobic development and that it is more active when oxygen is absent. In the presence of air, the yeast functions like a plant. It is nourished, respires and multiples. When placed in a reduced air supply, it gives up or suppresses almost completely its multiplication. From the alcoholic fermentation, it draws the energy which it needs. Then, the scarcer the oxygen, the slower the multiplication.

The experiments of one of Pasteur's students, Denys Cochin, indicate that fermentation does not take place in the total absence of oxygen. The yeasts are not strict anaerobic organisms but are intermediate between the aerobes and anaerobes. It is necessary for them to always have a little oxygen, and it may be said that every yeast that does not receive a minimum supply of oxygen from its ancestors or does not find it in the culture medium, will perish. Oxygen seems to have a beneficial action on the cellular activity and the secretion of enzymes.

These results have been contradicted to show that fermentation though favored by the absence of oxygen, is, moreover, accomplished in the scarcity of air (Brefeld, Hansen, Wehmer), but any invalidation of Pasteur's results seems not to have been produced up to the present time. More recently Palladine and Iraklionoff [1] have shown that if a yeast is able to produce small quantities of alcohol, even in the presence of air, it may be explained by assuming the presence of peroxidases which reduce peroxides, freeing nascent oxygen which may be active.

[1] Palladine, W., and Iraklionoff, P. La peroxydase et les pigments respiratoires chez les plantes. Rev. gén. de Bot. 23. 1911.

Prevalence of Alcoholic Fermentation

The phenomenon of alcoholic fermentation is not limited to the yeasts. Many of the molds[1] are also able to ferment the sugars. But this fermentation is less active and is more prolonged. Furthermore, it is only produced under certain conditions. A few examples taken from DuClaux will be given.

Among the molds, it is known that *Sterigmatocystis nigra,* which is exclusively aerobic, never produces alcoholic fermentation. Some of the other species, such as *Aspergillus glaucus* and *Penicillium glaucum,* are able to cause a slight fermentation. If, for example, some of the conidia of *Penicillium glaucum* are inoculated into a Pasteur flask containing a sugar medium, a well-developed mycelium is produced on the surface; after a time, the air becomes reduced in concentration and carbon dioxide accumulates with traces of alcohol. The amount of alcohol will always remain very small, and will scarcely pass from 1000th to 1500th of the total volume. Under the same conditions, *Aspergillus glaucum* will produce large amounts of alcohol. Pasteur has shown that in a culture of *Aspergillus glaucum* cultivated in 122 c.c. of beer wort for a year, 4.4 c.c. of alcohol were produced by a weight of yeast which scarcely surpassed 0.5 gram in the dry state. About seven times the weight of the plant in alcohol were produced.

Many other molds possess a fermenting action. *Mucor racemosus* will produce under the same conditions more alcohol than the two fungi mentioned above. According to Pasteur, the weight of alcohol will be 10 or 20 times the weight of the mycelium. *Mucor mucedo, circinelloides* and *erectus, Amylomyces rouxii* and *Aspergillus oryzae* are in the same category. With the molds, however, fermentation requires a longer time than the yeasts require to ferment the same amount of sugar.

Comparison of Intramolecular Respiration with Alcoholic Fermentation

As Pasteur has pointed out, alcoholic fermentation is not limited to the molds nor to the yeasts, but is carried on in all living cells which contain sugar. Indeed, alcoholic fermentation ought to be compared to what is called "intramolecular respiration."

Bérard demonstrated, for the first time in 1821, that fruits which were exposed to the sun absorbed oxygen and liberated carbon dioxide;

[4] Certain bacteria are also known which produce alcoholic fermentation.

in a word, they respired. But if placed in an atmosphere of limited oxygen supply, they quickly absorb this and continue to liberate carbon dioxide. Lechartier and Bellamy (1869) established the formation of alcohol under these conditions. This phenomenon remained unexplained until Pasteur undertook his experiments, when it was definitely proven to be an alcoholic fermentation. Pasteur placed plums under a flask filled with CO_2 and secured 6 grams of alcohol after 8 days. This same experiment was repeated on other tissues containing sugar. Muntz has been able to form alcohol by placing some of the higher fungi in a sugar solution. Maze, Goldewsky and Polszeniuz, and a few other authors, have secured similar results with certain plants. Green peas have the property, when placed under water away from air, of causing a sugar solution to ferment by simple contact, and act exactly as the yeasts except with less activity.

It seems, then, as if all cells which contain sugar are able, in the absence of oxygen, to function as yeast cells and produce alcoholic fermentation. We shall see that the yeasts, themselves, during inanition are able to produce a fermentation of their reserve glycogen, thus causing a sort of autofermentation.

"The alcoholic fermentation is not a characteristic inherent alone in the yeast cell nor a necessary manifestation for its existence. It is a characteristic variable with the conditions, but rather general. The yeasts differ from other plants only in the characteristic that they are able to adapt themselves better to anaerobic life and thus show this new phenomenon which is of so much industrial importance."

Differences in the Fermenting Function in Different Yeasts

If alcoholic fermentation is not a function special to the yeasts, one must not regard it as a specific characteristic. Many of the yeasts are not able to produce alcoholic fermentation but act only as oxidizing agents, as aerobes. Such are all of the *Mycoderma* and even the true yeasts producing endospores, as *Pichia hyalospora.* These form a luxuriant veil at the beginning of their development on carbohydrate media, which covers the surface of the liquid; they live then in contact with oxygen, consuming this gas and liberating carbon dioxide. Many of the *Torula*, although vegetating at the bottom of liquids, are also in the same class. Some of the other yeasts act like molds which we have just discussed. They live by preference in contact with air and possess only mediocre fermenting capacity. For example, the *Willia* and *Pichia* and the myco-yeast of Duclaux are such.

This yeast develops on liquid media with a typical veil or scum

which is folded in limited space and may become rather thick. In such a form the myco-yeast is a strong oxidizing agent and acts like an aerobic mold. It does not produce alcohol. If the veil or pellicle is transferred to a flask filled to the neck with a carbohydrate medium, an alcoholic fermentation results. From this moment, the myco-yeast acts like a yeast enzyme; its development is slow and almost the same weight is maintained which it had at the beginning. This yeast does not produce as much alcohol as ordinary yeasts. It never exceeds 3 per cent. *Monilia candida*, a fungus, intermediate between the yeasts and molds, acts in the same way. It produces a veil on the surface of the medium and grows aerobically; at the bottom of the flask it may appear as a deposit which decomposes the sugar. Hansen found that it yielded 1.1 per cent of alcohol in the time interval in which *S. cerevisiae* would yield 6 per cent.

Excepting these species, most yeasts, especially the industrial yeasts, are very energetic agents in alcoholic fermentation. These are distinguished from the myco-yeast and *Monilia candida* by the fact that they vegetate almost solely at the bottom of the culture flasks and form no veil at the surface. They almost always grow under conditions of restricted aeration and possess the ability to adapt themselves to anaerobic life which distinguishes them from other plants. These yeasts may then be regarded as true agents of alcoholic fermentation. We have stated above that the industrial yeasts may form about six times as much alcohol as the intermediate forms.

Fermentable Sugars

It is to the renowned researches of Fischer and Thierfelder that we owe our knowledge with regard to the laws which govern the fermentation of sugars. We have said a little about this, but it is of sufficient importance to receive more extended treatment.

From the investigations of these authors, it has been established that only those sugars are fermentable, in which the carbon atoms are in multiples of three. The series begins with glycerol ($C_3H_6O_3$), the tetroses not being fermentable, neither the pentoses. Finally come the hexoses which are very fermentable. These are made up of the dextroses, levuloses, fructoses, galactoses and mannoses. There is also a fermentable nonose ($C_9H_{18}O_9$); it is mannonose which is fermented by yeasts as easily as is glucose. After these come the bisaccharides ($C_{12}H_{22}O_{11}$) and the trisaccharides, melitrioses, or raffinoses; the melitrioses have the formula ($C_{18}H_{32}O_{16}$). The rule then seems to be general. There seem, however, to be certain exceptions. Thus it is that the fermentation of glycerose, always feeble,

has been contested by Emmerling. On the other hand, *Saccharomyces thermantitonum* ferments the pentoses (arabinose and xylose) and it seems to be true with *S. Ludwigii* according to Lindner. This author found that this yeast would ferment sorbose and tagatose and that *Sch. octosporus* would ferment xylose.

We have seen that yeasts are not able to ferment polysaccharides, but they are broken up by hydrolysis under the action of a special enzyme in each case. It is thus that starch is transformed by amylase to maltose, by maltase to glucose, saccharose into glucose and levulose by invertase, trehalose into glucose, and lactose into glucose and galactose, etc. Thus the sugars which have a more complicated structure are split into C_6 sugars by enzymes and these in their turn are decomposed into alcohol and carbon dioxide. Alcoholic fermentation offers, then, one of the best examples of molecular simplification which the enzymes are able to accomplish, and suggests the important rôle which these bodies play in cellular life. We have stated before that the α-methylglucosides and the β-methylglucosides are fermentable by certain yeasts after having been decomposed by maltase and methylglucosases.

It has also been established from the work of Thierfelder and Fischer that the sugars with C_6, C_{12} and C_{18} atoms are not fermentable to the same degree. Thus trehalose ferments more slowly and only by certain yeasts. Lactose is fermented only by a small number of yeasts.

The same thing is true with regard to the hexoses which are also not fermentable to the same degree. Among the ketohexoses, for example, levulose and d-fructose are alone fermentable, and among the d-glucoses only the d-glucose (grape sugar), d-mannose and d-galactose are fermentable.

According to Thierfelder and Fischer, a relation exists between the fermentability and the structure of the molecule. In other words, the enzymes have a direct relation to the stereochemic constitution of the molecule. This relation has been compared to that relation which exists between a key and its lock. The aldohexoses [1] suggest a very important example of this relation. Of the nine known aldohexoses, there are fermentable, as we have seen, only d-glucose, d-mannose and d-galactose with the last possessing much less fermenting possibilities than the other two. The chemical formulae of the aldohexoses are the same and differ only in their molecular grouping. Some of these will be given.

[1] The term aldohexoses refers to the hexoses which have an aldehyde group CHO, while the term ketohexoses refers to those which possess the keton group C=O.

HCO	HCO	HCO	COH	HCO
HCOH	OHCH	OHCH	HCOH	OHCH
OHCH	HCOH	OHCH	OHCH	OHCH
HCOH	OHCH	HCOH	OHCH	OHCH
HCOH	OHCH	HCOH	HCOH	HCOH
H_2COH	H_2COH	H_2COH	H_2COH	H_2COH
d-glucose	l-glucose	d-mannose	d-galactose	d-talose

This indicates the differences, although very slight, in the molecular arrangement of the molecules which has much effect on enzyme action. It is thus that d-glucose and l-glucose differ from one another only by the inverse position of their molecules. The stereochemical formula of one represents the image of the other as seen in a mirror. This structure is sufficient, however, to render d-glucose fermentable and to repress the fermentability of l-glucose. The differences between the grouping of d-galactose and d-talose are very slight. So slight are they that d-galactose is fermentable and d-talose is not.

Among the ketohexoses only d-fructose and levulose are fermentable.[1]

It is proper to add that the yeasts have a rôle in the fermentability of the hexoses. A true electivity has been established for certain sugars. Dubrunfaut has shown that, in a mixture of yeasts, one yeast will attack one hexose while another will decompose another hexose.

Formula of Alcoholic Fermentation and Secondary Products

Alcoholic fermentation consists in the transformation of sugars into carbon dioxide and ethyl alcohol. This change is accompanied by a liberation of heat, known for a long time in the fermentation of grape juice, and observed by Buchner in the fermentation induced by yeast juice. Bouffard has established a liberation of 20 to 23 calories for each 180 grams of sugar destroyed. A. Brown has obtained 21.4 calories.

Gay-Lussac has represented the alcoholic fermentation by the following simple equation

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$$

This equation does not take into consideration the heat exchange, for it is very difficult to express by such a simple formula a phenomenon of such complexity. It merely gives a general idea with regard to the change, but does not take into consideration the secondary products which are formed during the fermentation. Pasteur has

[1] Fischer, E. and Thierfelder, H. Berichte, 27, 1894.

shown that alcohol, carbon dioxide, glycerol, and succinic acid are all formed at the same time; however, these products are always in small quantities. According to this savant, 105.65 grams of glucose yielded the following:

Ethyl alcohol..	51.11
Carbon dioxide	49.42
Succinic acid	0.673
Glycerol	3.40

Among the products of fermentation may be found such secondary products as fatty acids, volatile acids, ethyl aldehyde, acetic acid, higher alcohols, ethers, and tyrosine and leucine. Some result from the decomposition of sugars while others are products of excretion from the cell. Glycerol seems to belong to the first category. The volatile acids are probably connected with the nitrogenous metabolism (Duclaux, Kruis). According to Ehrlich, it seems to be the same with succinic acid and the higher alcohols. It has been established by Ehrlich that leucine and isoleucine are assimilated by yeasts with the fixation of a molecule of water; they are decomposed into amyl and isoamyl alcohols by the liberation of ammonia which serves the metabolism of the yeasts. The ethers result from the action of acids formed by the action of air or other organisms with ethyl alcohol. As to the presence of aldehydes established by many investigators (Roux and Linossier, Duclaux, Roeser), they seem to result from the oxidation of alcohol by air or by the action of the yeasts. The observations of Trillat and Sauton, including those of Kayser and Demolom, have shown that wine yeast agitated in the presence of air caused a change of a part of the alcohol into aldehydes, ethyl and acetic. The acetic aldehyde is finally oxidized to acetic acid. It then seems as if the yeasts are able to use alcohol which results from fermentation and oxidize it to the aldehyde.

Character and Properties of Buchner's Zymase

What is the mechanism by which fermentation is accomplished? In 1858, Berthelot was the first to demonstrate that fermentation was brought about by enzymes secreted by yeasts. Bernard toward 1860 objected to this view. Pasteur and Denys Cochin had tried to isolate this enzyme from yeast but their efforts were in vain. Pasteur without discarding the possibility of an enzyme action thought more and more that fermentation was a vital act of the yeast cell itself.

It is known that the future has borne out Berthelot's contentions. Buchner, in 1897, succeeded in extracting the zymase from the yeast

cell. This discovery demonstrated that fermentation is able to be produced without the life of the yeast. This made it possible to abandon the vitalistic conception of alcoholic fermentation.

We have seen at the beginning of this chapter how Buchner extracted his yeast juice. It is definitely settled today that the juice contains a zymase and that its action is not due to particles of protoplasm as certain investigators believed in the beginning; when placed in contact with sugar it acts exactly like an enzyme.

It is not altered and continues to act in the presence of antiseptics (toluol and chloroform). On the other hand its efficiency is not entirely destroyed on filtration through a Chamberland bougie. Finally, Payen and Persoon have shown that yeast juice precipitated with alcohol gives a powder insoluble in water which possesses all of the properties of the juice. In order to secure this powder, the juice is precipitated by 12 times as much alcohol as there is juice. A mixture of 800 parts of alcohol and 400 parts of ether may also be used. The precipitate is filtered rapidly, washed with ether, and dried over sulfuric acid in a vacuum.

The investigations of Albert [1] and those of Rapp have discovered another method of securing alcoholic fermentation away from the living cell. These investigators have fixed the zymase in the cell by acetone which killed the cell, without altering the enzymes. The method consists of treating the yeast with from 10 to 20 times its volume of alcohol ether or acetone. All of the cells are killed. The yeast at first is rid of its water, is placed on a filter paper, washed with ether, and dried at 45°. A white powder is thus obtained made up of dead cells which has been called *zymine* or *durable yeast* (Dauerhefe). The cells which constitute this powder are endowed with the fermenting property like living yeasts and when they are put into a sugar solution, they induce fermentation immediately. When this powder is extracted by the method of Buchner, the juice thus secured possesses the same action as the living cells.

More recently Lebedeff [2] has obtained a very active zymase by the maceration of the yeast in water. For this, it is sufficient to macerate 2.5 to 3 parts of water with 1 part of yeast over a period of time. This is finally filtered through filter paper and a juice collected which is very clear and whose activity excels that secured by any of the other methods.

The quantity of zymase in living yeasts is variable. It is curious

[1] Albert, K. Herstellung von Dauerhefe mittels Aceton. Ber. d. chem. Ges. 31.

[2] Lebedeff, A. Extraction de la zymase par simple macération. Comp. Rend. Ac. des Sciences, 152, 1911.

to note that the quantity of zymase in pressed yeast increases considerably when the yeast is kept at low temperatures and that it diminishes in yeast during the course of fermentation. The investigations of Haydruck and Delbrück have shown that yeasts cultivated in a solution of sugars and mineral salts and removed at the moment when fermentation is most active, does not contain much zymase. If, on the contrary, a yeast is taken from a vigorous fermentation, washed, and kept at a low temperature, the zymase content increases rapidly. (Delbrück, Buchner, and Spitta.) All this seems to be explained by the fact that endotryptase and lipase find themselves affected by the low temperature and do not act on the zymase which they destroy under other conditions.

In the refrigerator zymase is kept with difficulty and soon loses its activity at the end of one or two days when placed at ordinary temperatures. At low temperatures, it is destroyed by degrees. This destruction was at first explained by thinking that zymase was easily oxidized by air. The investigations of Buchner and Antoni [1] have, on the contrary, indicated that oxygen has no action on zymase either during periods of its conservation or active fermentation, as they determined it. Its alteration arises from the endotryptase which is associated with it and perhaps to the lipase which one also finds in the juice. The work of Gromow and Grigoriew indicates that endotryptase attacks and digests it. On the other hand, the results secured by Harden, Buchner, Wroblewsky seem to indicate that lipase acts on the coferment. One is able, however, to keep yeast juice in all its activity by drying it in a vacuum at 35° C. The juice is then changed to a yellow which may be kept for a long time unaltered (10 or 12 months). Zymase also retains its activity for a long time if preserved in a 15 per cent solution of saccharose, this concentration acting on the endotryptase.

The investigations of the two English investigators, Harden and Young, have widened the horizon of our knowledge with regard to the constitution of zymase. They have shown that when yeast juice is introduced into a dialyzing apparatus, it may be divided into two parts, a non-dialyzable residue and a liquid which does dialyze. The residue is without fermenting activity and has been given the name of "inactive residue." The dialyzable liquid, which is without action on sugar, has been regarded as a *coferment.* Fermentation is only produced when the two parts are reunited. The inactive residue may also be regenerated by adding yeast juice which has been submitted to boiling, which indicates that the coferment is able to re-

[1] Buchner, E. and Antoni, W. Weitere Versuche über die zellfreie Gärung. Zeit. phys. Chem. 44, 1905.

sist boiling temperatures. The investigations of Buchner,[1] Hoffman, Duchacek,[1] Klatte,[2] Hoehn [3] and Resenbeck have confirmed the existence of a coferment. In adding this yeast juice to a double volume of boiled juice these investigators have noticed an increase in the activity, almost proportional to the amount of boiled juice added. On the other hand the addition of inactive boiled juice to the yeast juice, which had become inactive, restored the fermenting activity. This demonstrated that in old juice there is active zymase but that it lacks a coferment. This coferment seems, then, to disappear during fermentation before the zymase. Gromow and Grigoriew have also reported that if fresh zymase is added to a zymase which is becoming inactive, more fermentation is secured than if the fresh zymase was used alone. The old zymase has ceased to act on acccount of the alteration of its coferment and the addition of the fresh zymase regenerates it.

From all of this has been established that zymase may result from a mixture of *two* substances: a dialyzable body which resists boiling, the coferment, and a substance little or not dialyzable, which does not resist boiling. It is only by the union of the two bodies that fermentation takes place.

The investigations of Wroblewsky, Buchner, Harden and Young have permitted some explanations of the nature of the coferment. These authors have stated that the addition of phosphate salts of sodium or potassium will, like the ferment, produce an acceleration in fermentation. The addition of serum or lecithin produces the same effect. The coferment loses its activity by heating for 4 hours in water at 130° C. It is not attacked by trypsin but is destroyed by the lipase which exists in the yeast juice. The action of this lipase in the yeast juice seems then to be, with the endotryptase, the principal cause of the rapid loss of zymase activity. The lipase acts on the coferment and the endotryptase on the zymase. The coferment is present in lesser quantities than the zymase. It is able to be kept in sugar. This represses the action of proteolytic enzymes and perhaps the lipase. In this way, the action of these strong sugar solutions may be explained. Later on all these facts will be of much interest in the discussion of the mechanism of the alcoholic fermentation.

[1] Buchner, E. and Duchacek, E. Ueber fraktionierte Fällung des Hefepressaftes. Biochem. Zeitschr. 15, 1909.

[2] Buchner, E. and Klatte, F. Ueber das Koenzym in Hefepressaft. Biochem. Zeitschr. 19, 1909.

[3] Buchner, E. and Hoehn, H. Ueber das Spiel der Enzyme in Hefepressaft. Biochem. Zeitschr. 19, 1909.

The investigations of Buchner and his collaborators have revealed the presence of a substance in boiled juice which protected the zymase from the action of the endotryptase. This has received the name *antiprotease*. This enzyme protects gelatin and milk, also, from the action of the endotryptase in yeast juice. It prevents the liquefaction of milk and the peptonization of gelatin.

The existence of this antiprotease permits an explanation of how the fermenting action is preserved for many days when boiled juice is added to fresh juice; otherwise it would disappear rapidly. In the yeast juice, without the addition of the boiled juice, an almost complete disappearance of protein substances was noticed after 7 days. When boiled juice is added no precipitation of protein occurs. The addition of the boiled juice seems, then, to protect the fresh juice for a period of time against the proteolytic action of the endotryptase. The experiments of Buchner indicate that it will protect also from the action of pepsin and trypsin. This proves, then, that zymase is a protein substance. Careful experiments have indicated that it is possible to destroy the coferment of boiled juice without destroying the antiprotease. This may be accomplished by heating it for several hours. The juice thus treated still exerts a protective action towards gelatin and yeast juice. It contains, then, an antiprotease. On the contrary, this juice is not capable of regenerating the preserved yeast juice because it contains no coenzyme. The antiprotease is, then, distinct from the coenzyme. It does, however, have some likenesses to the latter; it is destroyed by lipase and seems to be a saponifiable ether of phosphoric acid. The antiprotease seems to play an important rôle in the life of the yeast and regulates its digestive functions (especially autolysis).

Zymase acts best in an alkalin medium. The addition of sodium carbonate and phosphate exerts a favorable action. It is destroyed by heating to 55°; in the dry condition, if desiccation has been carried out in vacuo at 40°, it is able to resist 140°. Temperature exerts a decided influence on the activity of zymase because the action of endotryptase and lipase on it is much altered with the temperature. That temperature at which zymase exerts the greatest fermenting action is about 14°. The optimum temperature seems to be higher; but endotryptase will attack zymase when the temperature is even higher.

Concentration plays a rôle in the activity of zymase. Fermentation increases with the concentration of the sugar; this explains the relation of concentrated sugar solutions (15 per cent) to the suppression of endotryptic action. The optimum concentration of sugar seems to be about 25 per cent.

The yeast juice contains, as we have said, hydrolytic enzymes for

carbohydrates (maltase, invertase). The hexoses are fermented immediately and the fermentable C_{12} and C_{18} sugars are transformed to hexoses by means of these enzymes. It contains also a glycogenase which allows it to induce the fermentation of glycogen upon which the living yeast has no action; the glycogenase is diffusible while the glycogen is not.

On the contrary, the ordinary juice does not act on lactose, but Buchner and Meisenheimer have been able to isolate from lactose-fermenting yeasts a lactase.

One is easily able to secure, by mixing some of Buchner's yeast juice with a solution of glucose, a fermentation which will start at the end of six minutes: 20 c.c. of yeast juice, with 8 grams of saccharose in the presence of 0.2 c.c. of toluol will yield after 96 hours from 0.7 to 1.87 grams of carbon dioxide. If these results are compared with those obtained with living yeast, the fermenting power of the juice seems feeble, for 1 gram of living yeast will produce from an 8 per cent solution of sugar at 40° in about 6 hours, 1.5 grams of CO_2. Zymase is not extracted in a pure state and it must be admitted that it makes up only a feeble part of the juice. However, in the fermentation with the living yeast, new zymase is formed constantly.

Among the secondary products, glycerol (3 to 8 per cent), traces of acetic acid and amyl alcohol, have been noticed. Lactic acid is often found but it may disappear and may be transitory. Further on, we shall see the significance of the formation of this lactic acid.

Mode of Action of Zymase

For a long time the following formula has expressed alcoholic fermentation

$$C_6H_{12}O_6 = 2(C_2H_5OH) + 2CO_2.$$

This equation has only general value. Among the two products expressed, there are many intermediate products. The determination of these has demanded the sagacity of the biochemists. It might be advisable to give some of the principal results which have been obtained.

After the work on oxidases and hydrogenases of yeasts Grüss [1] has put out a theory to explain the mechanism of alcoholic fermentation which is very interesting. This author regards glycogen as an intermediate product between fermentation and respiration. The polysaccharides will be decomposed into glucose by means of the hydrolytic enzymes (sucrase and maltase); this will be split into two

[1] Grüss, J. Zeitschr. f. ges. Brau. 27, 1904.

groups by the action of the zymase $\begin{matrix}CH_2OH\\|\\CH\ OH\\|\\COH\end{matrix}$. These groups combine with the protoplasm. The protoplasm will secrete glycogen which will finally be hydrolyzed into glucose by the glycogenase. Two conditions are then possible: when the yeast is in the presence of air and when it does not have air at its disposition.

In the first case, under the influence of oxygenase, put in evidence by Grüss, the molecules of glucose are decomposed into the above grouping which are oxidized by the oxygen liberated by oxygenase and thus changed into carbon dioxide and water. The reaction may be expressed by the following equation:

$$CH_2OH-CHOH-COH + 6O = 3CO_2 + 3H_2O.$$

This is ordinary aerobic respiration.

In the second case, the enzyme which we have come to know under the name of hydrogenase acts on the products of decomposition from glucose. Owing to the intervention of water, CO_2 will be formed in a first phase with a liberation of hydrogen. In the second phase these hydrogen atoms will be used to unite with the rest of the glucose molecule.

First Phase $CH_2OH-CHOH-COH + 3H_2O = 3CO_2 + 12H.$

Second Phase $2(CH_2OH-CHOH-COH) + 12H = 3C_2H_5OH + 3H_2O.$

This theory of Grüss, in spite of its complexity, has the advantage of explaining the rôle of oxygenase and hydrogenase.

Another theory has been expounded by Wohl. It has been stated that small quantities of lactic acid are often found among the products of alcoholic fermentation. Some, especially Buchner and Meisenheimer, have regarded this acid as intermediary in the mechanism of the decomposition of glucose into alcohol and carbon dioxide. This may be regarded as taking place in two phases. In the first phase, the zymase transforms the glucose into lactic acid, and in the second, another enzyme, the lactacidase, decomposes the lactic acid into alcohol and carbon dioxide. However, Buchner and Meisenheimer have not succeeded in transforming lactic acid into alcohol and CO_2 by yeast. This theory has been attacked by Slator, and Buchner himself has given it up. It may be regarded as of classic interest only.

One is not able to give up entirely the idea that a large molecule like glucose is not able to be split immediately into small fragments.

It ought to have intermediary products. Quite recently, Wohl and also Buchner,[1] Boyen-Jensen, and Fernbach[2] have admitted that this intermediate product may be dioxyacetone with the formula $CH_2OH–CO–CH_2OH$.

This compound, under certain rare conditions, is able to yield lactic acid. But, more often, this dioxyacetone will give alcohol and carbon dioxide directly. Alcoholic fermentation then acts in two phases: in the first, the glucose is transformed into dioxyacetone, and in the second phase, a dioxyacetonase will change the dioxyacetone into alcohol and carbon dioxide. Zymase will then in reality be made up of two enzymes acting successively.

Buchner and Meisenheimer have been able to obtain fermentation of dioxyacetone (2 per cent solution) by yeast juice. Lebedeff[3] quite recently obtained good results when making yeast juice ferment a 5 per cent solution of dioxyacetone. All of these facts are very interesting, but it still remains true that the demonstration of the formation of dioxyacetone during fermentation has not been accomplished. Its existence is, then, purely theoretical. We have cited the work of Harden[4] and Young, who have demonstrated that zymase is composed of two elements, one a dialyzable and thermostabile, the other not dialyzable and destroyed at 100° C. This last does not possess any fermenting function. If one adds to it the coenzyme, fermentation will result immediately.

But another agent has been found which will activate yeast juice which has no or little activity; it is the phosphates of either sodium or potassium. If a little soluble phosphate is added to yeast juice a brisk liberation of CO_2 results. This exists for a time proportional to the quantity of phosphate added, then it slows up and fermentation goes on as before. If phosphate is added again the phenomenon is repeated. One is thus able to reproduce it a number of times. The addition of phosphates then has the same effect as the addition of a coenzyme or the boiled inactive juice alone.

Such are the facts which the investigations of Harden and Young and Lebedeff have established. An ingenious conception of the mechanism of alcoholic fermentation has thus been formed.

[1] Buchner, E. La fermentation alcoolique des sucres. Rev. g. des Sciences, 21, 1910.

[2] Fernbach, A. Sur la dégradation des hydrates de carbonne. C. R. Acad. des Sciences, 150, 1910.

[3] Lebedeff, A. Sur le mécanisme de la fermentation alcoolique. Comp. Rendu Acad. Sciences, 153, 1911; Ueber Hexosphosphorsauerester. Biochem. Zeitschr. 27, 1910.

[4] Harden, A. Recherches récentes sur la fermentation alcoolique. Ann. de la Brasserie et de la Distillerie, 14th Year, 1911.

The phosphate added enters into a combination with the glucose. What demonstrates this is the fact that the phosphate is not able to be obtained again by the usual magnesium salt. Harden and Young have thought that the phosphate united to two half molecules of the hexose. The remaining half molecules, with three atoms of carbon, gave alcohol and carbon dioxide. The combination of hexose-phosphate is transformed by an enzyme *hexose-phosphatase* which re-forms a new combination with two remnants of the hexoses. The phosphate, then, follows a cycle and it is the quickness with which the phosphate traverses this cycle that determines the speed of fermentation.

Lebedeff has produced an important contribution in relation to this conception. He has been able to prepare the osazones, phenyl- and bromophenylhydrazones of this hexose-phosphate. He has isolated and analyzed the calcium salt. The following formula is given to this body $C_6H_{10}O_4(R_2PO_4)_2$, resulting from the condensation of two molecules of $C_3H_5O_2R_2PO_4$.

Young developed the following formula with either an aldehyde or ketone group free to form hydrazones:

$$\begin{array}{l} CHO \\ CH\text{–}PO_4H_2 \\ (CH\text{–}OH)_3 \\ CH\text{–}PO_4H_2 \end{array}$$

According to Lebedeff alcoholic fermentation takes place according to the following equations:

1. $C_6H_{12}O_6 = 2\ (C_3H_6O_3)$.
2. $2\ (C_3H_6O_3) + 2\ RHPO_4 = 2\ (C_3H_5O_2RPO_4) + 2\ H_2O$.
3. $2\ (C_3H_5O_2RPO_4) = C_6H_{10}O_4(RPO_4)_2$.
4. $C_6H_{10}O_4(RPO_4)_2 + H_2O = C_2H_5OH + CO_2 + C_3H_5O_2 + 2\ (RHPO_4)$

or even

5. $C_6H_{10}O_4(RPO_4)_2 + 2\ H_2O = 2\ (C_2H_5OH) + 2\ CO_2 + 2\ (RHPO_4)$.

This scheme involves the following:

Decomposition of the hexose into 2 molecules of triose (dioxyacetone).

Union of one molecule of the triose with 1 molecule of phosphate; a phosphoric ether results.

Condensation of two molecules of this phosphoric ether with one molecule of hexose-phosphate.

Decomposition of this hexose-phosphate into phosphate, alcohol and carbon dioxide.

This question, as we have seen, is extremely complex; the question is being studied but instead of becoming simpler becomes more complicated.

Lebedew [1] has later restated his idea of the mechanism of alcoholic fermentation as follows:

$$4\ C_6H_{12}O_6 = 8\ C_3H_6O_3.$$

Glyceraldehyde

$$4\ C_3H_6O_3 - 4\ H_2 = 4\ C_3H_4O_3.$$
$$4\ C_3H_4O_3 = 4\ C_2H_4O + 4\ CO_2.$$
$$4\ C_2H_4O + 4\ H_2 = 4\ C_2H_5{-}OH.$$

Dioxyacetone

$$4\ C_3H_6O_3 + 4\ RHPO_4 = 4\ C_3H_5O_2RPO_4 + 4\ H_2O.$$
$$4\ C_3H_5O_2RPO_4 = 2\ C_6H_{10}O_4(RPO_4)_2.$$
$$2\ C_6H_{10}O_4(RPO_4)_2 + 4\ H_2O = 2\ C_6H_{12}O_6 + 4\ RHPO_4.$$
$$2\ C_6H_{12}O_6 = 4\ C_3H_6O_3, \text{ etc.}$$

Neuberg [2] has studied the possibilities of intermediate compounds in alcoholic fermentation. He believes that the CO_2 which appears in alcoholic fermentation is split off from ($CH_3CO.COOH$) pyruvic acid. He found that it took from 2–8 seconds for CO_2 to be formed in yeast maceration + $CH_3CO.COOH$ while it took from 2–3 hours for it to be formed from yeast + glucose. Neuberg and his coworkers also used oxalacetic acid and found that yeast would change this to two molecules of CO_2 and one molecule of CH_3CHO. Hydroxypyruvic acid yielded CO_2 and glycoaldehyde, a ketobutyric acid yielded propionaldehyde and CO_2 along with some propyl alcohol. They find that the cleavage of pyruvic acid takes place 2000 times more rapidly than the complete fermentation of sugar. Neuberg and Kut then argue that two processes are involved in alcoholic fermentation. First, one class of enzymes hydrolyzes the C_6 molecule into C_3 chains. Secondly, another class of enzymes (carboxylase) breaks the C_3 chains up into C_2 and C_1 compounds. In further researches Neuberg has shown that yeasts possess an enzyme which decomposes pyruvic acid into acetaldehyde and CO_2. Acetaldehyde is readily reduced during fermentation to ethyl alcohol. From this it seems probable that aldehyde is an intermediate compound in fermentation. Neuberg and Reinfurth showed that if fermentation was carried out in the presence of Na_2SO_3 considerable amounts of aldehyde could be ob-

[1] Lebedew, A. and Griaznoff, N. Ueber den Mechanismus der alkoholischen Gärung. II. Chem. Berichte, 45 (1912), 3256–3272.

[2] See bibliographical index for complete list of Neuberg's publications on sugar-free fermentations.

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tained. The reactions illustrating the cleavage of glucose according to Neuberg's theory may be written as follows according to Euler and Lindner:

$C_6H_{12}O_6 - 2\ H_2O = C_6H_8O_4$ (Methylglyoxal-aldol)

$C_6H_8O_4 = 2\ CH_2{:}C(OH).COH$ or $2\ CH_3.CO.COH$ (Methylglyoxal)

$$\begin{array}{lll} CH_2{:}C(OH).COH + H_2O & H_2 & CH_2OH{-}CHOH{-}CH_2OH \\ & + \mid = & \text{Glycerol} \\ CH_2{:}C(OH).COH & O & CH_2{:}C(OH).COOH \\ & & \text{Lactic acid} \end{array}$$

$CH_3.CO.COOH = CO_2 + CH_3.COH$ (Acetaldehyde)

$$\begin{array}{lll} CH_3.CO.COH & O & CH_3.CO.COOH \text{ (Lactic acid)} \\ & + \mid = & \\ CH_3.COH & H_2 & CH_3.CH_2OH \text{ (Ethyl alcohol)} \end{array}$$

Neuberg [1] has produced more evidence to support his aldehyde theory of fermentation. By adding sodium sulfite, aldehyde and glycerol were the chief products.

Löb [2] has given the chemistry of alcoholic fermentation comprehensive study. He has produced much evidence on the fact that aldehyde is the important intermediate compound. He argues that the sugars tend to cleave into the same substances from which they may be built up. That aldehydes are intermediate in alcoholic fermentation has been stated by many, but few have gone far enough to produce either plausible evidence or experimental data to support their claims.

Kusserow [3] proposed that glucose was first reduced to sorbitol and this fermented.

General Theories of Alcoholic Fermentation

Alcoholic fermentation seems to have for its purpose the liberation of the energy necessary in the life of the yeast when it finds itself deprived of air under conditions in which respiration is not possible. This theory has not been accepted by all and it might be well to mention some of the different theories which have been put forth to explain this phenomenon.

Pasteur's Theory

Pasteur was the first to think that yeasts, when growing away from air, might seek the oxygen, which they needed, in the com-

[1] Neuberg, C., and Reinfurth, E. Natural and forced glycerol formation in alcoholic fermentation. Biochem. Zeit. 92, 234–66, 1918. Chem. Absts. 13 (1919), 2046.

[2] Löb, W. See bibliographical index.

[3] Kusserow, R. Eine neue Theorie der alkoholischen Gärung. Cent. Bakt. Abt. II., 26 (1910), 184–187.

pounds which were accessible to them. By decomposing these, they are able to get this oxygen. Alcoholic fermentation would then be a method for resisting suffocation. The yeast, not finding oxygen available and not being able to live without this element, will be obliged to take it from some of its combinations in sugar which they find in the medium.

The same thing takes place in other living beings. The yeasts possess, then, the property in common with other organisms, but they are better adapted than the others to produce fermentation and thus resist suffocation.

In short, states Pasteur, nearly all known beings without exception are only able to respire and sustain themselves by assimilating free gaseous oxgyen; there is a class, however, in which respiration will be quite active in order that they may live away from air by taking their oxygen from certain combinations, from which results a slow and progressive decomposition. This last class of organized beings will be made up of the ferments entirely similar to the beings of the first class, living with them, assimilating carbon, nitrogen and phosphates, having like them a need for oxygen, but differing from them in that they are able to respire with oxygen taken from compounds, when free oxygen is not available.

Pasteur's theory has precipitated numerous objections among which is this, that the classic formula of Gay-Lussac accepted by Pasteur does not leave a place for the setting free of oxygen.

This theory of Pasteur's has been modified. Alcoholic fermentation has always been considered as a phenomenon of resistance to suffocation and that it has for its purpose the securing of energy for the life of the yeast. The fermentation will be, then, from the viewpoint of energy, the equivalent of respiration. The yeast, during fermentation, continues to develop, making new tissue and retaining its usual functions. The yeast carries on a slow deliberate decomposition in quest of its energy and it is alcoholic fermentation which furnishes it. The yeasts are constructed to live in contact with or away from air. In the first case, they burn carbohydrates; in the other, they cause a breaking up or shifting of parts of a complex molecule. But the sources of cellular life are the same in each case.

Theory of Wortmann and Delbrück

Wortmann and later Delbrück have regarded alcoholic fermentation as a phenomenon comparable to the secretion of a toxin. In this case the alcohol serves the rôle of a poison with which the yeast is able to compete with other organisms with which it comes in con-

tact. The yeasts are able to withstand strong doses of alcohol, sufficient to kill other organisms. They are able to live in a medium which contains from 10 to 18 per cent of alcohol while other organisms are killed by from 4 to 10 per cent. Generally alcohol is not assimilated by yeasts and it is then useless to them.

The first yeasts, which were without doubt the wild yeasts, lived like other fungi in contact with air. But having taken on the ability of living in decaying fruits and in the mucous secretions of trees in order to secure sugar, they have competed with other organisms which lived under the same conditions. In this struggle for life, the yeast has been victorious against its adversaries and has survived, thanks to the fermenting function which constitutes a means of preservation for it. Yeasts are then adapted to live after a special manner away from air, secreting a large amount of alcohol which acts as a toxin.

The culture of yeasts by man has finally adapted them to anaerobic life and caused them to secrete increasingly large amounts of alcohol. According to this, the primitive yeasts were aerobic and slowly adapted themselves to anaerobic life.

Experience has demonstrated that if a new wine is exposed to the air, a vigorous growth of fungi takes place on the surface, consisting of *Botrytis*, *Penicillium*, *Dematium*, bacteria, and wild and cultivated yeasts. These organisms live along together for a time but as the yeasts produce alcohol, the medium becomes unfavorable for some of these fungi and the bacteria drop out. The wild yeasts are able to withstand these quantities of alcohol for a time but they in turn are killed as the concentration of alcohol increases. Finally, by means of their adaptation to alcohol, the cultivated yeasts are able to be triumphant over all of the various fungi which were present at first.

The phenomenon of alcoholic fermentation permits the yeasts to resist suffocation. By means of it they form alcohol and CO_2 and thus secure the heat which is necessary for their maintenance. Wortmann and Delbrück join the theory of Pasteur but their hypothesis has the merit of explaining the origin of alcoholic fermentation.

The theory of toxin formation has raised certain objectors who claim that the toxin is not generally secreted in sufficient quantity to injure the organisms which make it, while the yeast produces such quantities of alcohol that it is finally killed.

Theory which Makes Fermentation a Pnase of Respiration

Another theory, which seems to depend upon Pasteur's, is that making fermentation a phase of respiration. It rests upon the fre-

quency of the formation of alcohol in living tissue. We have seen that alcoholic fermentation is not a phenomenon exclusive to the yeast but is met among most fungi and also in tissues containing sugar. Alcohol seems to be rather frequently produced in cells. Berthelot, Devaux, and Maze have found alcohol in a great number of plants placed under normal conditions. Bechamp has isolated alcohol from the brains of sheep.

Therefore, from these contributions, certain authors think (Wortmann, Polszeniuz, Goldewski, Maze [1] and Duclaux, Pfeffer, Palladinn,[2] Stoklasa, etc.) that zymase exists in all organisms and functions in the usual manner. For these investigators, alcohol is always an intermediate product in respiration of plants and animals. Respiration, according to this, is made up of two phases. In the first, or intramolecular respiration, there is no need of concourse with oxygen; the sugar is decomposed into alcohol and carbon dioxide by zymase. This is intramolecular respiration or alcoholic fermentation. In the second phase, the alcohol, thus formed, will be changed in the presence of air by means of oxidase into carbon dioxide and water. This is respiration, properly speaking, or external respiration. In the absence of air, the phenomenon will stop with the formation of alcohol.

Thus, to express this theory with formulæ, the zymase would accomplish the following:

$$C_6H_{12}O_6 = 2\ C_2H_6O + 2\ CO_2.$$

The second phase which takes place in contact with air is as follows:

$$C_2H_6O + 3\ O_2 = 2\ CO_2 + 3\ H_2O.$$

In case that an organism, or yeast, finds itself away from air the change will stop at the first stage. As it goes on with much intensity, it will give sufficient energy for those organisms which are able to live without air like the yeasts. One finds here, then, a point of departure from Pasteur's theory.

Other authors go farther. They admit that alcohol is not only a product of respiration, but also term it a more simple assimilation of carbon. The hydrocarbon elements may then be transformed into alcohol before being assimilated. According to this, the hexoses which are formed from the polysaccharides by the various enzymes will be changed into alcohol by the zymase. If the phenomenon takes place in contact with air, a part of the alcohol is oxidized by the oxidase,

[1] Maze, P. La respiration des plantes vertes; théorie biochimique et théorie de la zymase. Rev. g. des sciences, 1906. No. 17.

[2] Palladinn, W. Sur la respiration des plantes. Biochem. Zeitschr. 18, 1909.

the enzyme of respiration, the other is utilized for the maintenance of the organism and the construction of new tissue. Zymase is then an enzyme of digestion the same as amylase, maltase, etc. If, on the contrary, the phenomenon takes place away from air some of the alcohol will remain unutilized, as is the case in the alcoholic fermentation by yeasts.

According to Duclaux, the term alcohol is not the simplest; by means of the oxidases according to him it may be changed into the aldehyde. Many have noticed the presence of alcohol in the product of fermentation. It is known that aldehydes are generally considered as the first step in the combination of H_2O and CO_2 in the green plant. The yeast acts, then, exactly as a higher plant. It assimilates hydrocarbon substances and forms formaldehyde as in the chlorophyll synthesis.

This theory is supported by a number of facts which are interesting enough to cite at this time. Thus, Laborde has called attention to a mold, *Allescheria Gayoni* (*Eurotiopsis Gayoni*), which produces zymase during a life very much more aerobic than that of the yeast and which always gives a little alcohol in the presence of air which, moreover, it uses for food. In Raulin's medium, in which alcohol is substituted for sugar, the fungus vegetates very easily. Alcohol is, then, a useful substance for it. It disappears in the form of water and carbon dioxide with a rapidity comparable to that of carbohydrates. The alcohol may be regarded as an intermediary product in the metabolism of the sugars. It is not apparent because it is used up as rapidly as it is formed. Quite recent experiments of Trillat and Sauton, as well as those of Kayser and Demlon, have shown that after the complete disappearance of sugar in wine, the yeast acts like any ordinary cell. In presence of air it respires like ordinary plants by oxidizing organic acids. It may oxidize the alcohol, and when agitated in the presence of air, ethyl or acetic aldehydes may be formed by this oxidation. It acts, then, like *Allescheria Gayoni* but in a more active manner. On the other hand it has been known for a long time that many of the *Mycoderma*, especially *Mycoderma vini*, and the mycoyeast of Duclaux, are capable of maintaining themselves at the expense of alcohol. A. Perrier[1] has encountered a certain number of microörganisms endowed with a considerable oxidizing power and in particular capable of developing in a mineral medium containing ethyl aldehyde as the source of carbon. This assembly of facts seems then to prove that alcohol and aldehyde are able to represent two stages in the assimilation of carbohydrates by plants.

[1] Perrier, A. Sur la combustion de l'aldéhyde éthylique par les végétaux inférieurs. Comp. Rend. Acad. Sciences, 151, 1910.

This theory has the advantage of explaining the liberation of carbon dioxide and the accumulation of alcohol during asphyxia of a plant deprived of oxygen.

However, numerous objections, in spite of the illustrations which have been presented, have been raised that alcohol is rarely a food for the yeasts. The oxidation of alcohol by fungi is not able to be regarded as a rare occurrence. According to certain authors, alcohol seems to be a waste product.[1]

Kostytschew [2] has noticed that when wheat grains are kept away from air and finally placed in air, alcohol is produced during the fermentation and not the oxide. Certain investigators have added to this theory a modification which resolves some of the difficulties. Thus Kostytschew, Boyen-Jensen,[3] and Blackmann [4] have admitted that alcohol produced by fermentation is not oxidizable. Alcohol will not be a normal product of respiration; it will form only when the intermediary products of respiration escape the action of oxydases. According to Boyen-Jensen and Blackmann the true intermediary product will be dioxyacetone. Kusserow [5] considers alcoholic fermentation in the light of incomplete respiration. With the exclusion of air the yeast reduces the sugar to a diatomic alcohol which further reduces to ethyl alcohol, carbon dioxide and hydrogen.

Autophagy or Autolysis of Yeasts

We should now investigate the curious phenomenon known as autophagy or autolysis. When, in a fermentation, the quantity of yeast is lower than 40 per cent by weight of the sugar, the fermentation stops immediately at the exhaustion of the sugar. But if, on the contrary, the quantity of yeast is greater than 40 per cent of the sugar by weight, the fermentation continues after the exhaustion of the sugar. The yeast lives, then, on its own substances. It ferments the glycogen which it has accumulated and accomplishes a sort of autodigestion.

This phenomenon may be observed in a yeast undergoing inani-

[1] Maquenne, L. La respiration des plantes vertes. Rev. g. des Sciences, 1905. 16th year. No. 13.

[2] Kostytschew, S. Ueber ein Einfluss ergorgener Zuckerlösungen auf die Atmung der Weizenkeime. Bioch. Zeitschr. 23, 1910.

[3] Boyen-Jensen, P. Die Zersetzung des Zuckers während des Respiration Prozesses. Ber. der deutschen Bot. Ges. 26, 1909.

[4] Blackmann, F. Assoc. Brit. pour l'Av. des Sciences. Congres de Scheefield, 1910.

[5] Kusserow, R. Respiration and fermentation, two allied physiological processes. Brennerei und Pressehefefabrik, 44 (1912), 1–3; Chemièal Abstracts, 7 (1913), 2237.

tion in water to which a little antiseptic has been added (creosote, toulol, phenol, etc.). The yeast is reduced to live at the expense of its glycogen which it has laid up in reserve and to attack its protein substances. Then, two phases may be distinguished in autophagy, first, the fermentation of glycogen and, secondly, the proteolysis of its own protein substances.

Glycogenase which is contained in the cells acts on the glycogen and causes a fermentation (autofermentation). The principal products formed under these conditions are alcohol, carbon dioxide, glycerol, and according to Salkowski, succinic acid. The fermentation of glycogen is then quite analogous to intramolecular respiration which is noticed in fruits containing sugar when placed away from air.

The yeast, at the same time, attacks albuminoid materials by means of its endotryptase and other proteases (guanase and arginase) which seem to play a very important rôle. The products of this digestion are nucleic bases, tyrosine, leucine, guanine, lysine, arginine, aspartic acid (Kutscher) and choline. Autophagy depends, according to Effront, not on the cells but on the enzymes which are formed in the cells when placed in inanition. In the presence of water the hydroxy compounds of carbon disappear with the liberation of carbon dioxide. The cells die rapidly at the end of about 6 days. In the presence of alcohol (7 per cent) and a little hydrofluoric acid, Effront[1] has been able to obtain a proteolysis of albuminoid substances; under these conditions the yeasts are easily able to support a denutrition without losing their fermenting power.

[1] Effront, J. Sur l'autophagy de la levure. Moniteur scientific, 1905, 16.

CHAPTER IV

PHYSIOLOGY OF YEASTS (CONTINUED)

Living Conditions of Yeasts. Their Relations to Their Environment. Parasitism and Symbiosis

IN this chapter we shall consider the living conditions of yeast, i.e., their habitat, their duration of life, the physico-chemical conditions which are necessary for their development, the influences which determine budding, the production of spores, and finally, the pathogenic yeasts and the question of symbiosis.

Habitat of Yeasts

Devoid of chlorophyll, the yeasts, like all other fungi, are unable to assimilate atmospheric carbon. They are, then, necessarily parasites or saprophytes. A certain number among them, such as the beer-yeasts and industrial yeasts, the cultivated yeasts, have been propagated from time immemorial by man. The greater part of them live saprophytically, especially when sugar is available. These are able to be regarded to a certain extent as domestic yeasts. In certain regions fruits make a good environment on account of their sugar content. However, a nectar may also be found in certain flowers (Berlese),[1] in the mucous secretions of trees (Ludwig, Hansen,[2] Lindner, Rose [3]) and rarely in the detritus from vegetable decomposition. It will be pointed out later on, that at the end of autumn, the yeasts are introduced into the soil by the fall of the fruits and the rains and there pass the winter.

The investigations of De Kruyff have shown that, contrary to the conditions in Europe, the yeasts in Java are very much more distributed on the leaves, both living and dead. The exceptional climatological conditions of this country, humidity and heat, favor this mode of life. Many *Torula*, *Mycoderma* and a few true yeasts have been

[1] Berlese, A. Verhalten der Sacch. an den Weinstocken. Rivista di pat. vegetali, 5, 1897.

[2] Hansen, E. C. Ueber die im Schleimflusse lebenden Bäume beobachten Mikroorganismen. Cent. Bakt. 5, 1889.

[3] Rose, L. Beiträge zur Kenntniss der Organismen im Eichenschleimfluss. Inaugural Dissert. Universität Berlin. 25 June, 1910.

found in the salt waters of the sea (Fischer and Brebeck).[1] They are frequently encountered in milk. (Maze, Dombrowski.) Finally many of the *Mycoderma* live in alcoholic beverages.

Many of the yeasts live as parasites in man and animals and cause quite varied lesions.

Duration of Life of Yeasts

The yeasts are capable of conserving themselves for a long time in the same media without perishing. Duclaux,[2] who has given this subject some attention, had the privilege of studying some of the cultures which Pasteur had used in his investigations on alcoholic fermentation. In 1885, after 5 or 6 years, in 15 attempts on old yeasts, he found only three which had died out. In 1889, after 11 to 17 years, out of 26 yeasts only 6 could not be revived. He has been able to observe living yeasts after 25 years. From this it will be seen that the yeasts are able to live for a long time in media in which the ordinary foods have been exhausted and to use materials which ordinarily would not be taken. Hansen found that yeasts could be kept for periods of from 13 to 17 years in a liquid containing 10 per cent of sucrose without acid.

Recent observations by Klöcker[3] at the laboratory in Carlsberg have shown that living cells of yeast were present in sucrose and beer wort solutions after 20 and 30 years. Will[4] has also found that yeasts would live for a long time in media such as beer wort. Among the cultures which were examined, the oldest was 18 years and 2 months.

Will[5] has published data on the longevity of yeasts under different conditions. He found that the yeasts would live longer in liquid gelatin because these remained moist longer. Even in dry cultures there would be living cells after a long time. Meissner[6] secured some

[1] Brebeck, B. and Fischer, B. Zur morph. Biol. und Syst. d. Kahmpilze, Jena, 1891.

[2] Duclaux, E. Traité de Microbiologie, Fermentation alcoolique 3, Paris, 1900.

[3] Klöcker, A. Fermentation organisms. III. Conservation of fermentation organisms in different nutritive media. Comp. Rend. trav. lab. Carlsberg, 11 (1917), 297–311.

[4] Will, H. Noch einige Mitteilungen über das Vorkommen von lebens- und vermehrungsfähigen Zellen in alten Kulturen von Sprosspilzen. Cent. Bakt. 48 (1917), 35–41.

[5] Will, H. The persistence of living yeast on gelatin cultures. Cent. Bakt. Abt. II, 31, 436–453; Beobachtungen über das Vorkommen lebens- und vermehrungsfähigen Zellen in sehr alten Würzkulturen. Cent. Bakt. Abt. II, 44, 1916, 58–75.

[6] Meissner, R. Ten-year experiment on the longevity of wine yeasts in pure cultures on 10 per cent cane sugar solutions. Zeit. Gärungsphysiologie, 1, 106–113.

interesting data on the longevity of yeasts. He used 25 pure cultures grown in 10 per cent cane sugar solutions without renewal. Fifteen of these yeasts retained their vitality for $10\frac{1}{4}$ years, while nine of them died after eight and one-half years. One remained alive. Gayon and Dubourg [1] made an investigation which bears indirectly on this subject. They examined wines made in 1810, 1818, 1819, 1832, 1836 and 1846 and found living yeasts capable of causing alcoholic fermentation.

ACTION OF PHYSICAL AGENTS ON THE YEASTS

Temperature

Moist yeasts die generally between 50° and 55° C. some being able to withstand 60° (Hansen and Kayser[2]). In the dry state, they resist more elevated temperatures. Certain are able to withstand, without perishing, a temperature of from 100 to 110°, others from 115 to 120°. The ascospores are much more resistant to heat and generally withstand a temperature which is about 5° higher than the vegetative cell. (Kayser.) On the other hand, the investigations of Pictet and Young indicate that the yeasts are capable of resisting very intense cold. These investigators have submitted yeasts to temperatures of 130° below zero for 24 hours without killing them. Doemus has stated that the yeast of Frohberg could resist temperatures of − 150° for from 5 to 20 minutes. Cochran and Perkins [3] investigated the effect of high temperatures on yeast. They heated their yeasts in a syrup and found that 58° C. for 30 minutes did not stop fermentation; 65° C. for the same length of time caused a devitalization of the yeast so that fermentation was reduced. The yeasts were killed at 70° C. Wells [4] found that the thermal death point in yeasts is considerably effected by the presence of certain substances. Starch and sugars were found to raise it. He states that the approximate thermal death point in bread is about 68° C. It is quite well known, however, that bread may contain living cells since, during the baking process, the temperature is not high enough to kill all of the yeasts.

[1] Gayon and Dubourg. Experiments on the vitality of yeasts. Rev. vit. 38, 5-8.

[2] Kayser, E. Action de la chaleur sur les levures. Ann. Inst. Past. 3, 1889.

[3] Cochran, C. B. and Perkins, J. H. The effect of high temperatures on yeast. Jour. Ind. Eng. Chem. 6 (1914) 480.

[4] Wells, E. P. The thermal death point in yeast. Vermont Agricultural Experiment Station, Bull. 203, 1917.

Light

Light does not seem to possess any marked action on yeasts. However, Marshall Ward [1] noticed a destructive action of light on the ascospores of *S. Pyriformis*. It will be pointed out that the different rays of the spectrum have an accelerating or retarding influence on the sporulation of yeasts. According to Martinand yeasts are destroyed by an exposure of 4 hours to the sun's rays at 40 to 45°. An exposure of 3 days at 36° produces the same effect. The absence of light, on the contrary, over a long time does not effect the yeasts. Although light acts on the vitality of the yeasts, it does not seem that its effect is very great, and it may be generally said that yeasts are resistant to the action of light.

The recent investigations of Buchta [2] made with *Saccharomyces cerevisiae* and *Ludwigii* have shown that diffuse daylight stopped the budding of yeasts. The cells not exposed to it multiplied almost twice as fast as those which were exposed. Electric light had the same influence. When cultures of yeasts were placed at different distances from the source of light, those which were farthest away multiplied most quickly. The blue light had a more marked action than the red which did not seem to effect the budding. The infra-red rays did not seem to impede budding. The ultra-violet rays, however, exhibited a marked action, and an exposure of 10 seconds sufficed to stop budding. A longer time resulted in the death of the cells. Von Recklinghausen [3] found that it took 300 seconds' exposure at a distance of 200 mm. from a quartz lamp burning 66 volts and 3.5 amperes to kill yeast cell.

Jacquemin and Giurel [4] found that radioactive emanations exerted a favorable action on fermentation. A radioactivity of $\frac{1}{2}$ to 1 unit per liter exerted a favorable action on the splitting of sugars.

Moisture

Yeasts need moisture; they resist drying easily as the experiments of Hansen [5] have shown.

[1] Ward, H. M. The ginger beer plant and the organisms composing it. Philos. Trans. Royal Soc., 1898, 183.

[2] Buchta, L. Ueber den Einfluss des Lichtes auf die Sprossung der Hefe. Cent. Bakt. Abt. II. 41 (1914), 340.

[3] Von Recklinghausen, M. Purification of water by the ultraviolet rays. Jour. Amer. Water Works Assn. 1 (1914), 565–588.

[4] Jacquemin, G. and Giurel, G. The influence of radioactive emanations on yeasts and alcoholic fermentations. Bull. Agr. Intelligence, 5 (1914), 1505.

[5] Hansen, E. C. Recherche sur la morph. et la physiol. des ferments alcooliques. Comp. Rend. des trav. de Carlsberg, 4, 1898.

Metals and Salts

Zikes [1] found that aluminum had a slightly stimulating effect on the fermenting and regenerative function. Bokorny [2] studied the effect of certain uncommon salts. RB_2SO_4 and Cs_2SO_4 in the presence of potassium salts favored the development of yeast. Lithium salts proved injurious to yeast propagation An increase of over 0.1 per cent of the potassium phosphates in a medium is not advantageous. Two per cent $(NH_4)_2SO_4$ does not seem to hinder the development of yeast. Bokorny,[3] in another paper, has reported a very complete study of the action of metallic salts. Practically all of the metallic salts were studied. Kossowicz [4] has shown that yeasts liberate iodin from KI-mineral-sugar solutions.

Bokorny [5] has stated that manganese is not poisonous to yeasts if it is in the form of its salts. Budding took place when yeast was put into 1 per cent solution of $MnSO_4$, while in a 3 or 5 per cent solution budding was stopped. This is attributed to the fact that, unlike the other metals, manganese does not unite with the protoplasm. Boas [6] studied the effect of arsenic compounds on yeast. He found that, at first, sodium metaarsenite and potassium and sodium arsenate had a repressing action which was eventually overcome if the yeast was kept in contact with these solutions for a period of time. Low temperatures were said to increase the intensity of the poisoning action but without killing the yeast. Mitra [7] found that the chloride of sodium, potassium, calcium and magnesium are more or less toxic to yeasts in concentrations. KCl was the least and NaCl the most harmful.

[1] Zikes, H. Influence of aluminum on yeast and beer. Allgem. Zeit. Bierbrau. Malzfabr. 41, 71–4, 83–7.

[2] Bokorny, Th. The influence of cesium, rubidium, and lithium salts on yeast in comparison with the action of potassium and ammonium. Allg. Braw. Hopfen-Ztg. 52, 1469–70.

[3] Bokorny, Th. Action of salts of metals upon yeast and other fungi. Cent. Bakt. Abt. II., 35, 118–197.

[4] Kossowicz, A. and Loew, W. The behavior of bacteria, yeasts and molds towards iodin compounds. Z. Gärungsphysiologie, 2, 1913.

[5] Bokorny, Th. The non-poisonous properties of manganese. Chem. Ztg. 38, 1290.

[6] Boas, F. Action of arsenic compounds on yeast. Chemical Abstracts, 12 (1918), 1101.

[7] Mitra, S. K. Toxic and antagonistic effects of salts on wine yeasts. Calif. Pub. Agr. Sci. (15) 3, 63–102, 1917.

Pressure

The investigations of Regnard [1] and Melsens [2] have shown that the yeasts are able to resist a very strong pressure. Regnard submitted a yeast to a pressure of 1000 atmospheres for 1 hour without killing it. Melsens has used pressures of 8000 atmospheres and noticed no diminution in the vitality of the yeasts so treated. Hite, Giddings and Weakley [3] in studying the effects of high pressures on microorganisms used a number of common yeasts. Samples of grape sugar in water which were inoculated with baker's (Fleischman's) yeast did not ferment after being subjected to 60,000 pounds pressure per square inch for a half hour at room temperature. With *Saccharomyces cerevisiae*, Meyer, there was no growth after 80,000 pounds pressure. While the results are not concordant, it may be seen that this yeast could stand pressures of between 50,000 and 55,000 lbs. pressure for 10 or 20 minutes, in distilled water. In 3 per cent cane sugar solution, there was one instance where this yeast resisted 60,000 pounds for 10 minutes. *Saccharomyces albicans*, Reess, in one instance, resisted 60,000 pounds pressure for 10 minutes. Choplin and Tammann,[4] as quoted by Hite and his colleagues, have stated that yeasts could resist a pressure of 3000 kilograms per square centimeter (43,000 pounds per square inch).

Antiseptics

Nowak [5] found that ozonization had a detrimental effect on the multiplication of yeast. It was brought out that this method may be used to remove undesirable bacteria from yeast cultures. Lindner and Grouven [6] employed four disinfectants towards yeast. These were corrosive sublimate (ammonium), fluoride, formalin, and antiformin. Will and Wieninger [7] experimented with ozone on yeast.

[1] Regnard, P. Influence de la pression sur la levure. C. R. Ac. des Sciences, 98, 1884.

[2] Melsens. Comp. Rend. Ac. des Sci. 1870.

[3] Hite, B. H., Giddings, N. J., and Weakley, C. E. The effect of pressure on certain microörganisms encountered in the preservation of fruits and vegetables. Bulletin 146, West Virginia University Agricultural Experiment Station, 1914.

[4] Choplin, G. W. and Tammann, G. Ueber den Einfluss hoher Eindruck an Mikroorganismen. Zeit. Hygiene, 45 (1903), 171.

[5] Nowak, C. A. Influence of ozone on yeast and bacteria. Jour. Ind. Eng. Chem. 5, 668, 1913.

[6] Lindner, P. and Grouven, O. What influence has an increase in the quantity of yeast on the disinfecting power of various antiseptics. Wochschr. Brau. 30, 133–135.

[7] Will, H. and Wieninger. Zeit. f. d. ges. Brau. 1910.

They found that 0.56 gram in a cubic centimeter of air was toxic for 30,000,000 cells.

Euler and Emberg [1] have stated that the hydrogen centration influences the development of bottom yeast. This is to be expected since other forms of microörganisms act in the same way.

Physiological Conditions of Budding

Budding is accomplished each time the cell finds itself in a suitable environment with no deterring factors, such as the accumulation of products of metabolism. Aeration plays an important rôle; it seems to accelerate budding. However, it is not indispensable. Hansen [2] has noticed that budding took place in the presence of nitrogen with no oxygen present. It is also known that budding continues during alcoholic fermentation. It is known, to the contrary, that sporulation does demand the presence of oxygen. Sporulation and budding then differ on this point which closely separates them.

Temperature exerts a preponderant influence on budding which is a function of temperature. Hoyer has calculated, for example, that in a medium of gelatin, *S. Pastorianus* formed a new generation at 13° every 6 hours while at 35° the budding was accomplished in about 3 hours. The experiments of Hansen have shown that there exist minimum, optimum and maximum temperatures for every species of yeasts. Hansen has determined the limiting temperatures for 11 species of yeasts (*S. cerevisiae, Pastorianus, intermedius, validus, ellipsoideus, turbidans, Marxianus, Willia anomala, Pichia, membranaefaciens, Saccharomycodes, Ludwigii*). He found that the maximum temperatures of these species varied from 47° to 34° C., the minimum temperature from 0.5° to 0.3° C.

Particular Types of Budding

It has been stated above that yeasts may grow as a sediment at the bottom of the culture flasks (anaerobic life), or as a scum or veil (aerobic life). The formation of the scum generally represents a particular type of budding.

[1] Euler, H. and Emberg, F. The sensitiveness of living yeast to hydrogen and hydroxyl-ion concentration. Zeit. Biol 69, 349–64 1919. Chem. Absts. 13 (1919) 2691.

[2] Hansen, E. C. Recherches sur la physiol. et la morphologie des alcooliques ferments XI. La spore de Saccharomyces devenue sporange. Recherches comparatives sur les conditions de croissance vegetative et le development des organes de reproduction des levures et des moisissures. C. R. du lab. de Carlsb. 5, Book 2, 1902.

Certain yeasts, such as the *Mycoderma* (*Mycoderma vini* and *cerevisiae*), are essentially aerobes, never producing fermentations and forming on the surface of the media a scum which is very characteristic, reminding one of fungi. This scum is gray and dry. Later, it develops and becomes wrinkled. Many bubbles of air are found retained between the cells. But the *Mycoderma* are not characteristic yeasts; they do not form spores and their place in a classification is quite uncertain.

Hansen has distinguished two groups among the *Saccharomycetes* or true yeasts. In one, which includes *Willia* and *Pichia*, the scum appears very rapidly at the beginning of the culture. It is well developed, dry, and filled with globules of air which are retained between the cells. This is the characteristic scum for the *Mycoderma*. For this group, the scum is a normal method of vegetation. It is understood, then, that budding is confused with the formation of the scum.

In the other group, to which belong the majority of known species, a scum may or may not be formed. When one is formed it appears at the end of fermentation and under conditions which Hansen has well established. On the other hand, this scum differs in the group.

It is necessary, in order for the scum to form, that the surface of the medium be quiet and in direct contact with air. Hansen has recommended that a 24-hour culture be used to which there is added new wine. The culture should be shaken, after which a drop is carried over to a flask half full of new wine and closed with a cap of paper. At the end of a shorter or longer time, the principal fermentation is finished and on the surface of the medium are seen little spots of yeast. These remain isolated as little islands, until they join to form a thin scum which is gray and mucous. If the flask is shaken, parts of the scum break off and fall to the bottom; eventually a new scum will form over the surface. During the formation of this scum, the medium becomes a clear yellow. The scum, thus formed, differs markedly from that formed by *Mycoderma;* it is less tenacious and has a more viscous appearance.

Other yeasts do not form scums but simply rings about the side of the tube. With some both are formed.

The formation of the scum is influenced by the temperature, as the investigations of Hansen have determined. Hansen has shown that certain limits of temperature exist outside of which the scum is not able to form. Between these limits the time of formation is determined by the temperature. The time is constant for a given temperature. A certain optimum temperature, variable with each species, allows the most rapid formation of the scum. It is an interesting fact to note that there is no relation between the temperature at which scum for-

mation goes on most rapidly and budding. Budding is less dependent on the temperature.

In determining these various temperatures and the time necessary for the scum to form in six species (*S. cerevisiae*, *Pastorianus*, *intermedius*, *validus*, *ellipsoideus*, and *turbidans*), Hansen noticed that scum formation was slower at low temperatures than at high temperatures. On the other hand, it may be stated that the maximum temperature for scum formation is lower than the maximum for budding. The temperature limits of scum formation vary with the species and furnish important characteristics for the differentiation of these species.

Physiological Conditions of Sporulation

We shall see, in a later chapter, that sporulation is generally a function of inanition of the yeast. It is often necessary that the yeasts have accumulated from former culture media the reserve products necessary for the formation of ascospores. Under these conditions, the cells begin to bud as soon as these reserve products are exhausted. It seems from all this that the formation of ascospores is determined exclusively by the lack of food. This is the conclusion to which the investigations of Klebs [1] leads us. However, it has been known for a long time that the yeasts are able to sporulate very rapidly on certain solid media (gelatin added to wine, slices of carrot or potato) and sometimes in liquid media during fermentation. Sporulation seems, then, to have other causes. Klebs admits that in the case of solid media, such as nutrient gelatin or slices of carrot, if the yeasts are able to sporulate, it is only those cells which are in the innermost parts of the colonies where they are prevented from using the medium. These find themselves in bad conditions of food supply which explains their sporulation. In these colonies, the cells occupying the marginal portion of the colony will continue to bud and multiply while the cells which are on the inside of the colony will be reduced to conditions which favor the formation of spores.

The investigations of Hansen,[2] to which we owe much of our information with regard to sporulation, have demonstrated, on the contrary, that this matter is much more complicated. If the lack of food is one of the most important factors, it is by no means indispensible. Indeed, Hansen has stated that contrary to the ideas of Klebs, in cultures on gelatin or beer wort, the ascospores form in the

[1] Klebs, C. Allgemeine Betrachtungen. Jahrb. wissenschaft. Bot. 35, 1900.

[2] Hansen, E. C. Recherches sur la physiologie et la morphologie des ferments alcooliques. II. Les ascospores chez le genre Saccharomyces. III. Sur les Torula de M. Pasteur. IV. Maladies provoquées dans la bière par les ferments alcooliques. C. R. du lab. de Carlsberg, 1, 1883, 5, 1902.

cells on the margin as well as in the cells inside of the colonies. The lack of food, then, has little bearing in this case because the well-nourished cells also form ascospores.

According to Hansen, two factors seem to determine sporulation: the lack of food and the accumulation in the medium of toxic excretion of the yeast cell. With the yeasts which are placed on gypsum blocks or in distilled water, it is the lack of food which is probably the reason for sporulation. With yeasts cultivated on solid media (slices of carrots, or nutrient gelatin) it is the action of toxic excretions which arrests budding and causes sporulation. It is the same reason which causes some yeasts to form ascospores in a fermenting solution. The alcohol may hinder budding and provoke sporulation. Hansen has shown, however, that certain chemical substances, such as saturated calcium sulfate, are capable of stopping budding and producing sporulation.

In recent investigations by Saito, the rôle of toxic substances of the yeast in relation to sporulation has been studied. According to this author, only the cells on the periphery of a colony sporulate. It seems to be a question of the amount of food. Saito thinks that the deprivation of food is the main factor inducing sporulation but that *Schizosaccharomyces octosporus* is an exception to this rule.

But these two factors, the lack of food and the accumulation of toxic products, are not sufficient in themselves to determine the formation of spores. The investigations of Hansen and Barker[1] have shown that there are a number of secondary conditions which are necessary: free access of air, temperature, humidity and condition of the cells. More recent researches by Purvis and Warwick have shown that light exercises an influence on sporulation. We shall now take up successively these various conditions.

A. Condition of the Cells. In order for a cell to sporulate it is necessary that the cells be young and vigorous and that they have accumulated, either from former culture media or from the medium in which they are taken, a reserve of products necessary for the formation of ascospores. We have seen, indeed, that cells destined to form ascospores, have accumulated metachromatic corpuscles, fats and glycogen which are finally absorbed by the ascospores during their formation. The media in which yeasts are placed are of much importance in relation to sporulation. Hansen reported that dextrose had a favorable influence on sporulation in *Saccharomyces Ludwigii*, and Klöcker has reported the same observation for certain *Pichia;* these yeasts only formed ascospores after a preliminary cultivation in beer

[1] Barker, P. On the spore formation among the Saccharomycetes. Jour. of the Federated Institutes of Brewing, 8, 1902.

wort to which dextrose had been added and others to which alcohol had been added.

The recent investigations of Saito [1] have furnished an explanation of some of these facts and given information with regard to the formation of ascospores which have been overlooked up to the present time. Certain yeasts when placed in an environment with little food do not contain certain necessary chemical substances which vary with the yeast. For *Zygosaccharomyces mandshuricus* which has been the special object of Saito's investigations, these substances are dextrose, levulose, galactose, sorbose, raffinose, mannite, dulcite, sorbite and glycerol. These substances seem to exercise a stimulating effect on the sporogenic function. There seemed to be a minimum concentration for each of these compounds for sporulation. For example, the minimum concentration of dextrose and levulose for *Zygosaccharomyces mandshuricus* was between 0.125 and 0.25 per cent; for galactose, raffinose, and glycerol between 0.25 and 0.50 per cent, and for sorbose and dulcite between 0.5 and 1 per cent. The addition of small amounts of potassium phosphate and peptone exercised a favorable action on sporulation in *Zygosaccharomyces mandshuricus*. The salts of sodium, potassium, magnesium and carbon had a favorable action.

Some substances, such as beer wort to which gelatin had been added and decoction of koji, had a stronger action on the production of ascs than the carbohydrates. Indeed, the action of beer wort and decoction of koji caused a large number of ascs, but among them were some with no ascospores. These various substances seem, then, to have a specific action not only on the production of ascs but also on the formation of ascospores and their maturation.

Aside from substances which stimulate the formation of ascospores, there are substances which, in a marked manner, retard sporulation. The salts of ammonia have such an action and when yeasts are placed in certain concentrations of these salts, although all other factors are favorable for sporulation, they do not sporulate. In general it seems that those yeasts in which the asc is preceded by a sexual process sporulate under more complex conditions than those which are parthenogenetic.

Sartory [2] has noticed a symbiosis between a yeast and bacterium. The yeast sporulated only in association with the bacterium. Zetlin [3]

[1] Saito, K. Untersuchungen über die chemischen Bedingungen für die Entwicklung der Fortpflanzorgane beinieigen Hefen. J. College of Sci. Imperial Univ. Tokyo, 39, 1916.

[2] Sartory, A. Sporulation d'une levure sous l'influence d'une bactérie. Comp. Rend. Soc. Biol. 72, 1912.

[3] Zetlin, Sophie. Influence of previous nourishment upon spore formation in yeast. Chemical Abstracts, 8 (1914), 3807.

studied the effect of certain foods on spore formation. It was found that ammonium sulfate, asparagin, glycocoll and peptone had a favorable action. Spore formation was greatly increased. The results with different sugars were variable. Some favored spore formation while others tended to repress it.

B. Influence of Air. Another indispensable condition is a free access of air. Hansen has demonstrated this by the following example. Some young cells of *S. cerevisiae* and *S. Pastorianus* are inoculated into a Freudenreich flask containing a little water (about 5 drops in each flask), and deprived of air. A first lot of these flasks is placed into a bell jar with a little alkaline pyrogallol and from which the air has been sucked out as far as possible. Another lot is placed under another bell jar in contact with air. Both are placed at 25° C. After six days the lots should be examined and it will be noticed that the yeast cells in the first lot will contain no ascospores while the cells in the second lot will contain a large number. If now the flasks of the first lot be exposed to air an abundant crop of ascospores will be noticed after a few days. Thus the lack of air inhibits sporulation and the access of air is indispensable to the formation of ascospores. On the other hand it is the oxygen of the air which is so indispensable to the formation of ascospores. Hansen has demonstrated this by using nitrogen as the atmosphere and under these conditions much less sporulation was secured.

Then, oxygen is an indispensable factor for the formation of ascospores. Sporulation, in this regard, acts in a very different manner than budding which, as we have stated, is able to go on in the absence of oxygen.

C. Temperature. One of the factors essential to sporulation is temperature. The investigations of Hansen have shown that, for each variety of yeast, there exist certain temperature limits outside of which sporulation becomes impossible. Between these limits, the time necessary for the formation of ascospores is constant for a variety for a given temperature. Outside of these temperatures, there are others more or less favorable at which ascospores form after varying lengths of time. Hansen has shown that we may put down for each variety:

First. The temperature limits which allow the formation of ascospores. A maximum and minimum.

Second. The optimum temperatures at which ascospores appear most rapidly.

Third. Temperatures which are between these limits and at which the time of ascospore formation is more or less long, depending on how far it is removed from the optimum.

In determining these three temperatures for 6 varieties of yeasts (*S. cerevisiae, Pastorianus, intermedius, validus, ellipsoideus* and *turbidans*) Hansen has noticed that sporulation is dependent upon three laws which are able to be announced as follows:

First. Sporulation is accomplished slowly at low temperature but increases with the rise in temperature up to a certain optimum; above this temperature ascospore formation becomes slower and slower until it finally ceases completely.[1]

Second. The most favorable temperature for six varieties of yeasts was about 25°.

The temperature limits of these yeasts with regard to sporulation are situated between 0.5 and 37.5° C. It is interesting to note that, as for the formation of the scum, the temperature limits for sporulation are included in the limits of budding. The minimum temperature for sporulation is not as low as that for budding and the maximum is not so high. Sporulation in order to be accomplished requires a temperature more pronounced than budding. It seems that the higher the temperature for budding, the higher is the maximum temperature for sporulation. The experiments of Hansen indicate, however, that there is no parallelism between the two temperature curves for budding and sporulation.

The observations of Hansen mentioned above, as well as those of many other investigators as Nielsen, Klöcker, and many others, on maximum and minimum temperature limits, have confirmed this. Nevertheless, some species are able to attain maximum temperatures of 40–41°. Sometimes they are situated around 15° C. as in certain *Pichia* studied by Klöcker. With regard to optimum temperatures, they range around 25°. These temperatures and the times required for ascospore formation vary with the yeast. Hansen has been able to establish very important characteristics for the differentiation of species.

The action of temperature has been mentioned by Saito in relation to formation of the asc in certain yeasts. Saito isolated a *Zygosaccharomyces* which, depending on the temperature, formed ascs derived from a copulation, or parthenogenetically. On slices of carrot on which the yeast germinates very actively, the ascs were formed by a copulation at 25 to 27° C. At 33°–39° C., on the contrary, the ascs were produced by a parthenogenesis.

[1] Herzog has shown that the curves which show this phenomenon resemble those of Tamman on the influence of temperature on diastatic action; these reach a maximum and decrease progressively. According to the same author, they also agree with van't Hoff's law that the speed of a chemical reaction is a function of the temperature.

D. Humidity. Naegeli has argued that the principal factor in sporulation is desiccation and that this phenomenon is brought about only in cells which are partly dried. The investigations of Hansen, on the contrary, have shown that humidity is an important, if not indispensable, condition of sporulation. It is easy to show this by Hansen's own experiment.

Blocks of plaster of Paris are prepared and then plunged into water in order to soak them; on each of these, a little of the yeast is placed and the blocks placed in dishes without water, covered with a glass plate. Some other blocks are placed in dishes covered with a filter paper and still containing no water. Another series of blocks are placed in dishes with water and covered with a glass plate. In a few days it will be seen that the yeasts in the dishes with water have formed numerous ascospores; those in dishes without water and covered with the glass plate have a few; those in the dishes covered by a filter paper have still less.

The evaporation of water hindered the formation of ascospores. Humidity is then necessary for sporulation. Evaporation does not completely stop the formation of spores, for a few cells will sporulate on the blocks undergoing evaporation. Thus, we are able to explain sporulation in yeasts in nature on fruits; in the superficial layers of the soil they are capable of sporulating in spite of the absence of humidity.

E. Light. According to the investigations of Purvis and Warwick [1] the rays of certain wave lengths have an influence on sporulation. By placing moist plaster of Paris blocks in dishes, the walls of which were covered with different colors, they were able to establish the following facts:

1. The red rays of longer wave length accelerate the formation of ascospores which appear more rapidly than in the presence of white light. They also seem to be more favorable to sporulation than obscurity and seem to stimulate sporulation.

2. The green rays seem to retard the formation of ascospores.

3. The blue or violet rays retard sporulation more effectively than the green.

4. Finally, the ultra-violet rays have a pronounced retarding action; they seem to have a bad effect on the vitality of the cells.

These results are able to be explained on a chemical basis, for it is well known that the rays of short wave length have a greater chemical activity than the long wave lengths. Also it might be regarded that the former determine the chemical modifications in the cell which

[1] Purvis, E. and Warwick, R. The influence of spectral colors on the sporulation of Saccharomyces. Proceedings of the Cambridge Society, 14, 1907.

are unfavorable to the formation of ascospores, while the rays with longer wave lengths, having a lower chemical energy, have little influence and permit the formation of ascospores.

Acid or Alkaline Media: The investigations of Saito have shown that the degree of acidity or alkalinity determines sporulation and an increase in acidity or alkalinity is accompanied by a retardation in the formation of ascospores. The higher limits of acidity for *Zygosaccharomyces Mandshuricus* on plaster blocks are 0.5–1.0 per cent of sulfuric acid, malic acid, tartaric or citric acid. The higher limits of alkalinity are 0.2 to 0.4 per cent of sodium hydroxide. Certain toxic substances also affect the sporulation.

Osmotic Action: This also exerts an effect on sporulation. The maximum concentration for spore formation in a yeast depends upon the species. For an osmophilic species like *Zygosaccharomyces Mandshuricus* the concentration is high. In a substrate with 25 per cent of salt this yeast still sporulates. The investigations of the action of other salts towards this yeast give data which depend upon the nature of the salt. Thus, a very concentrated solution of potassium nitrate has much less effect than isotonic solutions of NaCl. On the other hand, the method of using the substance also determines the results.

Parasitism of the Yeasts. Pathogenic Properties. Symbiosis

Quite a number of the yeasts are parasites. They seem to have received less attention than the other vegetable parasites. It is only with animals and man that the parasitism in the yeasts is of any importance. *Endomyces albicans*, a fungus related to the yeasts, has been known for a long time to cause lesions in man. Remack has found in the intestines of mammals a true yeast capable of sporulation which received the name of *Saccharomycopsis guttulatus.* Metschnikoff in 1884 discovered in the general cavity of a crustacean (Daphnia) a yeast which caused a special infection. It was by means of this yeast, on account of the thin wall of the daphnia, that Metschnikoff discovered phagocytosis. The yeast was called *Monospora cuspidata.*

Other pathogenic yeasts have been observed in other animals. Bütschli and Dangeard have found them in the Anguillula, Schaudin in the intestines of Culex and Lindner in the larvae of the fly *Corethra plumicorum.*

For a dozen years the number of pathogenic yeasts has been notably increased by the discovery of different ones which cause various troubles in man and animals under the name of blastomycosis. Rivolts and Micellone have described the *Cryptococcus farciminosus* which causes farcy in the horse. Raynaurd, Lucet and Guegen

have described the *Cr. linguae-pilosae* which causes a malady in man. Achalme and Troissier found *S. anguinae* which caused an angina. According to Le Dantec certain dysenteries may be caused by yeasts. Quite a number of yeasts have been described in tumors. One of the most characteristic of these is the yeast of Curtis, *Saccharomyces subcutaneous tumefaciens*, which is a true yeast forming ascospores. Blanchard, Schwartz and Binot have described a yeast which caused a tumor. Vuillemin and Legrain have isolated *S. granulatus* which had pathogenic properties.

The frequency of pathogenic yeasts has lead certain authors to attribute to these fungi a varied rôle in disease, especially for some of those diseases for which bacteria have not been discovered. Thus attempts have been made to explain rabies and cancer on the basis of the presence of yeasts. The possible relation of yeasts to cancer has held the attention of bacteriologists and a brief résumé of the subject will be presented although the subject has been abandoned today and is of historical interest only. The presence of yeasts (Curtis, Blanchard, Schwartz and Binot, Vuillemin [1] and Legrain) in many tumors has suggested that possibly these organisms were the cause.

This idea has been supported especially by Russel who observed, in a large number of carcinomas, spherical bodies which he called yeasts. The investigations of Corselliet, Frisco, Plimmer, and Bra seem, at first thought, to confirm this opinion. These investigators isolated a yeast from many tumors (sarcomas, epitheliomas and carcinomas). Plimmer especially found *Cr. Plimmeri* in more than one thousand carcinomas. On the other hand San Felice pretended to have provoked the formation of true neoplasms by animal inoculation of a yeast isolated from certain fruits, the *Cr. neoformans*. For a time this pathogenic theory of yeasts for cancer held a very strong position among clinicians and anatomo-pathologists.

It is generally agreed that among all of the published observations, there is not one which will stand close scrutiny and which is sufficiently demonstrative. It is now known that the bodies which Russel observed are only degenerate cytoplasm. On the other hand, Roncali, Plimmer and Bra have run afoul in their animal inoculation experiments, for the yeasts which were isolated never reproduced the tumors.

If certain investigators, among others Carselli and Fisco, San Felice, have been able to produce true tumors by inoculation of yeasts, it was never demonstrated that the tumors had any histolog-

[1] Vuillemin, P. Cancer et tumeurs végétales, Bull. des séances de la Soc. des Sc. de Nancy, 1900. Les Blastomycètes. Rev. gén. des Sciences, 1901, No. 16.

ical similarities to cancer. The inoculation and rapid increase *in loco* of these yeasts alone determine the common lesions exactly as in all foreign bodies. According to Maffuci and Sirleo, many yeasts, which have been isolated from malignant tumors, came from the air and not from the diseased tissue. These authors have been able to isolate yeasts from numerous carcinomas and sarcomas,[1] but they also obtained them by exposing gelatin plates to the air of the laboratory.

It then seems probable that many of the yeasts, said to have been isolated from cancer tissue, really came from the air.[2]

It seems certain, however, that yeasts may be found in certain malignant tumors, but they must be of only secondary importance in the disease. They never cause it. The yeasts develop simply because they find the organism weak and in the neoplastic tissue a favorable environment — a good culture medium. One is able, for example, to secure *Endomyces albicans* in certain tumors which, as is generally known, causes an infection in infants, aged and generally "run-down" individuals. The blastomycelial theory of cancer has been definitely rejected.

Pathogenic Properties of Yeasts

Even though it is admitted today that yeasts have no relation to cancer, it is possible to inquire whether certain yeasts, either special yeasts or common saprophytes, are not capable of presenting, at times, toxic or pathogenic properties. Generally speaking, the question has been answered in the negative, and it is now recognized that the yeasts are almost without significance for the higher animals.

Rabinowitch,[3] in inoculating 50 varieties of ordinary yeasts into various animals, found only seven which were pathogenic for the mouse and rabbit. None caused even the slightest reaction in the guinea pig. The animals which were killed seemed to be dead from infection and not intoxication. Yeasts do not seem to secrete a toxin which has any action on animals.

The results of investigations conducted by Skchiwan [4] have shown that yeasts have practically no chemiatric action towards leucocytes. This was demonstrated by introducing *S. pastorianus* in capillary tubes into the peritoneum of guinea pigs and rabbits.

[1] Guegen, F. Les Champignons Parasites de l'homme et des animaux. Thèse d'agrég. de Pharmacie, Paris, 1902.

[2] Guiart, J. Précis de parasitologie. Baillierie édit. Paris, 1910.

[3] Rabinowitch, L. Untersuchungen über pathogene Hefearten. Zeitschr. Hyg. 21, 1896.

[4] Skchiwan. Ann. Inst. Past. 8, 1890.

By injecting into the peritoneal cavity of a guinea pig a culture of *S. pastorianus* and examining what happened, by removing a little of the peritoneal fluid at regular intervals, the same author,[1] observed a phagocytosis of the yeasts. They were demonstrated to be alive, however, by means of inoculation. After from 2 to 3 hours they were broken up in the interior of the leucocytes and at the end of from 10 to 11 days they were proven to be dead by means of inoculations into beer wort. If, however, in place of an ordinary yeast, a pathogenic yeast be used, such as *S. subcutaneous tumefaciens*, the same phenomena are observed with the single difference that phagocytosis is more energetic. This commences after a sort of lag, at first by the polynuclear leucocytes and finally the mononuclears; often a cell is observed which has been ingested by many leucocytes. On the other hand, a yeast may defend itself by surrounding itself by a mucilaginous envelope. A battle between the yeasts and the leucocytes ensues. But finally the leucocytes triumph and at the end of two or three days all the yeast cells find themselves ingested.

It may be inferred, then, that ordinary yeasts do not exhibit a pathogenic phase and that pathogenic yeasts themselves provoke only a light of doubtful intoxication. According to Casagrandi,[2] and Demme [3] a yeast, *Crypt. ruber*, caused an acute enteritis in young infants. The active agent was probably a secondary cleavage product from the milk in which it was secured.

If one summarizes the numerous observations of secondary infections by yeasts and the diverse lesions of the skin, mucous membranes or internal organs, the cases are rare where these fungi exhibit any actual pathogenic rôle. Blastomycoses thus seem to be exceptional diseases and not so very frequent.[4]

That we drink with wine large numbers of yeasts might indicate their harmlessness. For a long time we have attributed curative properties to beer yeasts toward such infections as furunculosis. Sergent [5] has noticed an antiseptic action of yeasts against infections with *Staph. pyogenes aureus*. Perhaps these properties find their explanation in the existence of a toxin recently demonstrated by Hayduck, Fernbach and Vulqium. It has been shown in the preceding chapter that, according to certain authors, yeasts secrete a toxin endowed

[1] Skchiwan, Ann. Past. Inst. 13, 1899.

[2] Casagrandi, O. Saccharomyces ruber. Ann. d'Ig. sperm. 1898. Vols. 7 and 8.

[3] Demme, R. Saccharomyces ruber. Ann. de microg. 1889 and Annali d'Ig. sperm. 1897, Vol. 7.

[4] Duval and Laederich. Arch. de Protistology 3.

[5] Sergent, E. Levure de bière et suppuration Ann. Inst. Past. 17, 1903.

with a decided bactericidal power.[1] The investigations of Neumayer and Anderson seem to indicate that the yeasts are able to withstand the action of the digestive juices and may thus pass through the digestive canal. Hawk and his colleagues at Jefferson Medical College have reported on the value of yeasts in the treatment of furunculosis. They claimed better results than were secured by the use of autogenous vaccines. The use of yeasts in therapeutics is not a new idea. In the earliest of times they were used against the pyogenic cocci.

Symbiosis of Yeasts

Symbiosis is the association of two different organisms which live together, both being benefited. It seems that yeasts are able to undergo similar associations.

Thus it is that in certain industrial yeasts made up of different fermenting agents there is a living together or a symbiosis.[2] Will has reported the case of two varieties of yeast which have functioned in a brewery for 12 years without any noticeable change in their individual characteristics. A sort of equilibrium seems to have been established between the two varieties which permits them to live together without harming each other. Schönfeld has cited a similar case of a little brewery in which the leaven for four years gave a rapid clarification with feeble alternation. This leaven was composed of two yeasts, one which had low alternation and the other with higher alternation. These two species lived for two years in close contact without harm and preserved their relative strengths. Van Laer has also noticed a case of equilibrium among yeasts in the innoculum of a top fermentation and which for a long time lived in symbiotic relations. In this, two yeasts predominated; first, a yeast of the type *cerevisiae* which caused saccharose and maltose to ferment; secondly, a *Torula A* which caused saccharose to ferment, but which acted on maltose a little and which gave the beer an agreeable taste and odor. Two other varieties were present to a lesser

[1] The use of yeasts in nutrition has received some attention. Voelz and Baudrexel (Ann. de la Brasserie et de la Distillerie, 1911) have shown, by a series of experiments with dogs, that yeasts constitute a good source of nitrogen. At the suggestion of Professor Delbrück, a dozen assistants at the Fermentation Institute at Berlin have replaced, for several weeks, a part of their meat at breakfast by 20 gms. of dried yeast. None of them suffered any trouble by the introduction of this new food (Delbrück, La Levure, un noble champignon, 1st International Congress of Brewing, Brussels, 1910). Völtz (Biochem. Zeit. 93, 101–5) has stated that yeast should not be fed in the living condition if it is to be of food value.

[2] These examples are taken from Duclaux, Traité de microbiologie.

proportion, two yeasts of the *Pastorianus* type; one A, was rather inactive, the other B seemed to take part in the secondary fermentation. But it is necessary to say that such associations in yeasts are rare. In most of the breweries where mixtures of yeasts are employed, it is exceptional that an equilibrium is maintained between them. One almost always predominates over the others.

Certain fermentations in distilleries produced by mixtures of fungi also seem to be cases of symbiosis. The fungi transform the starch into maltose which the yeasts ferment. A large number of fermented beverages used in different countries, resulting from the fermentation of starch, seem to be due to such symbiotic associations of yeasts and fungi. For example, Saké, an alcoholic drink prepared by the fermentation of rice, and used in Japan, is an example. This beverage is obtained by the action of different organisms, among which is *Aspergillus oryzae* and many yeasts and molds. The starch of the rice is changed into maltose by *Aspergillus oryzae* and this sugar fermented by the molds and yeasts. Arrack, obtained by the fermentation of molasses and rice flour, is produced by an agent made up of bacteria, molds (*Chlamydomucor oryzae, Rhyzopus oryzae*) and two yeasts. The fermentation in bread seems to result by the symbiotic action of a yeast and bacteria.

Another example of the same order has been mentioned by Lutz.[1] According to this author, tiby, an alcoholic drink of the Mexicans, is produced by the action of a yeast, *Pichia Radaisia*, and a bacterium *B. mexicanus*, which live in symbiotic association. The yeast living in contact with air is not able to induce fermentation. Associated with the bacillus it brings about the alcoholic fermentation. The bacterium plays its only rôle in keeping the concentration of oxygen down. Lutz has been able to bring about an experimental symbiosis with *P. Radaisia* and *B. subtilis*.

Another classic example of symbiosis has been observed by Freudenreich[2] in kefir, the fermented milk. He has isolated 4 microorganisms: 1, the kefir yeast; 2, *Streptococcus a* which coagulates the milk; 3, *Streptococcus b* which possesses probably a lactase; and 4, *Dispora caucasica* or *Bacillus caucasicus* whose rôle is not known. According to Freudenreich, the yeast does not possess a lactase and is thus unable to ferment lactose. This is accomplished by *Streptococcus b*. This is, then, a symbiotic association. Jörgensen has observed another yeast in kefir, *S. fragilis*, which possesses a lactase and is thus

[1] Lutz, L. Association symbiotique du Sacch. Radaisii. Bull. de la soc. myc. de France, 1906.

[2] Freudenreich, E. Bakteriologische Untersuchungen über den Kefir. Cent. Bakt. 3, 1897.

able to ferment lactose. Beijerinck has also confirmed this. This author found *S. Kephir*.

Sartory [1] has recently described a yeast which sporulated only in the presence of a bacterium with which it lives.

Rist and Khoury,[2] on the other hand, have observed a similar phenomenon in the fermentation of leben, an Egyptian fermented milk. This fermentation is due to two yeasts, the *S. lebenis* and *Mycoderma lebenis*, and a bacterium *Streptococcus lebenis*. The two yeasts alone are not able to ferment the lactose. But, associated with *Streptococcus lebenis*, they bring about the fermentation of the milk. The bacterium seems, then, to act on the lactose in some way to make it available for the yeasts.

Another very curious example of symbiosis is furnished by the parasites in that rare infection in man known as "black tongue." Guegen's [3] work seems to indicate that this disease is caused by a mold *Oospora lingualis* and a yeast *Cryptococcus linguae-pilosae*. The yeast functions only when it is in association with the mold. These observations have been confirmed by the investigations of Thaon.[4]

Carpano [5] has reported that, in infections by *Cryptococcus farciminosus*, *Staph. pyogenes aureus* and *Strept. adenitis equi* are found. Possibly this is another symbiotic relationship.

It may be possible to have symbiotic relations between certain yeasts and the cholera vibrio, for Metschnikoff has shown that this latter organism is favored by the presence of a *Torula*.

One is able to cite many examples in which two forms of life are able to live together. However, up to the present time, we have only observed symbioses between two yeasts or between a yeast and a bacterium. It is possible to have symbiosis between two totally different organisms, such as a yeast and an animal. Thus, Lindner [6] found *Saccharomyces apiculatus parasiticus* in an hemoptera, *Aspidiotus Nerii*, in which it was always present without seeming to exer-

[1] Sartory, A. Sporulation d'une levure sous l'influence d'une bactérie. Comp. Rend. des Soc. Biol. 72, 1912.

[2] Rist, E. and Khoury, J. Etude sur un lait fermenté comestible. Cent. Bakt. and Leben d'Egypte. Ann. Past. Inst. 1902, 16.

[3] Guegen, F. Sur Oospora lingualis et Cryptococcus lingualis. Arch. de Parasit. 1909, 12.

[4] Thaon, P. Symbiose de levure et Oospora dans un cas de langue noire. Soc. de Biologie, 67. 1909.

[5] Carpano, M. The association of bacteria in Cryptococcus farciminosus infection. Ann. Ig (Rome) 28, 273–279. 1918.

[6] Lindner, P. Das Vorkommen der parasitischen Apiculatus Hefe in auf Hefen schmarotzenden Schildläusen und deren mutmassliche Bedeutung der Nonneraupe. Wochen. f. Brauerei, No. 3, 1907.

cise any pathogenic rôle. Conte and Faucheron [1] have also observed yeasts in the fatty tissue of female coccidia. They were led to regard this observation as a sort of symbiosis.

This hypothesis has been confirmed by the investigations of Pierantoni and Karl Sulc which, on account of their great biological interest, are important enough to mention here. All of the authors who have studied the adipose tissue of the homoptera have noticed the existence in these tissues of special organs which have received different names, depending on the insects in which they have been observed, but which seem to have a certain similarity; such are the pseudo-vitellius and green bodies described by Huxley, Lubhock, Balbiani and Henneguy in various Aphides, the *polar mass* observed by Heymons in the eggs of the *Cicadides* and the *oval body* found by Berlese in the genus *Dactylopius*. The significance of these organs has remained unknown until the present time. However it has been stated that they were cells with contents of fat droplets or protein grains. Some have stated that they served to control the reserve products.

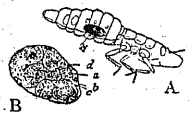


Fig. 53. — A, Larva of *Ptyelus lineatus* with mycetome *M*. B, Section of mycetome of *Ptyelus lineatus* (after Karel Sulc).

The work of Pierantoni and Sulc, carried out independently at the same time, have shown that these organs, which appear to be in all of the homoptera, present the same structure and may be homologous.

These organs to the number of two in each insect are situated on each side of the intestines of the insect quite near the reproductive organs (Fig. 53, A). They are made up of a mass of large cells with a yellow or greenish epithelial membrane. They contain an ameboid nucleus and a cytoplasm filled with small spherical or oval bodies. These bodies, regarded by some as reserve products (fat or proteins), in reality resemble the yeasts. These yeasts vary from one insect to another. Budding yeasts are especially found, among which is a variety already mentioned, *Saccharomyces apiculatus parasiticus*, *Schizosaccharomyces* and some yeasts related to these latter to which Sulc gave the name of *Cicadomyces*.

These yeasts agree with those described by Lindner, Conte and Faucheron in the Coccidia and are encountered constantly, probably being handed down in the egg. The cells situated in the center of these organs divide regularly, and cause by their growth a shattering of the superficial layers which liberates the yeasts. These happen to come in contact with the ovaries and penetrate the egg during the

[1] Conte, A. and Faucheron, L. Présence de levures dans le corps adipeux de divers Coccides. Comp. Rend. Acad. des Sciences, 144, 1907.

process of its formation. They form a little mass at one of the poles made up of a few cells. This mass is soon surrounded by a number of cells resulting from the segmentation of the reproductive vesicles and of blastodermic origin. This polar mass remains distinct from all of the other organs during the development of the embryo. It is placed in the rear part of the abdomen of the growing cells, the yeasts penetrating into their interior until the mass parts into two bodies which take positions on both sides of the intestines as described above.

Sulc and Pierantoni have been then led to conclude that the pseudo-vitellius, green body or oval body constitutes organs resulting from a sort of inflammation produced by these yeasts on the blastodermic cells in the course of segmentation. Sulc has designated them as *mycetomes*, reserving the name *mycetocytes* for the same cells filled with yeasts.

Both of these investigators admit that the yeast found in the mycetomes is living in symbiotic relation with the insect. At first the yeast was probably a parasite but a continued association with the insect brought about a symbiotic association. According to Sulc the mycetomes play a similar rôle to that of the lymphatic ganglions. They protect the insect from the invasion of bacteria, by means of the yeasts which are contained in their interiors, for it has been shown by the investigations of Haydruck and Fernbach that they are germicidal. On the contrary, Pierantoni [1] believes that the mycetomes play a rôle in digestion. The homoptera live on vegetable sap and take in much starch and sugar. Part of these hydrocarbons is assimilated, the rest remaining in the intestinal tract to be finally eliminated. This author thinks that the yeasts in the mycetomes serve in the assimilation of the carbohydrates and in the transformation to carbon dioxide and alcohol. This question apparently needs further study.

Vandevelde [2] has paid considerable attention to the question of symbiosis in yeast. He has carried rather extensive investigations. When certain yeasts fermented together, the fermentation was carried on to a further degree. The conclusions which this author draws from his investigations are that mixed yeasts give better results than pure cultures in the fermentation industries. It is interesting to

[1] Pierantoni, U. L'origin di alcuni organi d'Icerza perchasi e la simbiosi eredaria. Bull. Soc. Napoli, 23, 1909; Origine e struttura del corpo ovale del Bactylopius citri e del corpo verde dell' Aphis brassicae. Ibid. 24, 1910. Ulteriori osservazioni sulla simbiosiereditaria degli omotteri. Zool. Auz. 36, 1910.

[2] Vandevelde, A. J. J. Chemical phenomena in the symbiosis of yeasts. Rev. Gen. Chim. 18 (1915), 88–95; On symbiotic life of yeast races. Original Communications, 8th International Congress of Applied Chemistry, 14 (1912) 191–202.

wonder whether symbiosis in yeasts may not be explained in part on the basis of vitamines, as has been suggested by certain pieces of research for the bacteria.

Vitamines in Yeast: The importance of "accessory substances," the so-called "vitamines," in the treatment of certain deficiency diseases, has caused investigators to examine different substances for them. While our knowledge, with regard to vitamines, seems to be in a transitory state, it may be advisable to mention a few of the more important papers on the presence of them in yeasts. Funk [1] isolated 2.5 grains of vitamine fraction from 100 kg. of dried yeast. When this was injected in the muscle of a pigeon suffering from polyneuritis, complete recovery followed. This original substance was further fractionated. Some of these were thought, at that time, to be nicotinic acid. Seidell [2] found that brewers' yeast was the cheapest as well as the richest source of vitamines. The yeast cells are dried hydraulically and allowed to autolyze at 37.5° C. for 48 hours. After cooling, the liquid is filtered. In this clear filtrate will be found about 50 per cent of the raw material, and 23 per cent of the total solids. One cc. of this injected into a paralyzed pigeon caused relief in an hour and a return to normal condition in 12 hours. Seidell [3] stated later that the autolytic process influenced the power of the vitamine. Emmett and McKim [4] found that the yeast vitamine of Seidell should be accompanied by vitamine containing foods in order to accomplish normal gains in weight and complete recovery. Hawk [5] and his colleagues, in another connection, have found that yeasts are decidedly beneficial for treating skin diseases. Improved conditions resulted in many cases from ingestion of yeast, where autogenous vaccines caused no relief. In many cases there was a general improvement in the condition of the patient "quite unassociated . . . with the particular disease in question." This might indicate that some essential or beneficial substance was added to the diet through the ingestion of the yeast.

That vitamines may be necessary for the development of yeast,

[1] Funk C. Studies on Beri-beri. Further facts concerning the chemistry of the vitamine fraction from yeast. Brit. Med. J. 1913, I, 814. J. Physiol. 46, 173–9.

[2] Seidell, A. Vitamines and nutritional diseases. Public Health Reports, 31, 364–70, 1916.

[3] Seidell, A. The vitaminic content of brewers' yeast. Jour. Biol. Chem. 29, 145–54, 1917.

[4] Emmett, A. D. and McKim, L. H. The value of the yeast vitamine fraction as a supplement to a rice diet. J. Biol. Chem. 32, 409–19, 1917.

[5] Hawk, P. B. *et al.* The use of bakers' yeast in the diseases of the skin and of the gastrointestinal tract. Jour. Amer. Med. Assn. 69 (1917), 1243–1247.

seems to be indicated by the investigations of Williams [1] and Bachmann.[2] Williams presents data to show that water-soluble vitamine may be necessary for the growth of yeasts themselves. Williams used the development of a single cell of yeasts as the indication of the presence or absence of vitamine. Substances which were known to be rich in vitamine were also found to be rich in the yeast growth-promoting substance. Williams believes that these substances which stimulate yeast development are the same as those which prevent beri-beri. In a synthetic solution alone, a cell of yeast developed very slowly. Under identically the same conditions, with the exception of the addition of one part in 60,000 of growth-promoting substance, many more cells were formed from the single cell. Bachmann found that water-soluble B vitamine was quite necessary for vigorous development of a yeast isolated from canned pears. Both papers bear out the contention of Wildier,[3] an earlier worker, who found that some substance, to which he gave the name " bios," was necessary for vigorous development of yeasts.

[1] Williams, R. J. 1919. The Vitamine Requirement of Yeast. A simple biological test for vitamines. J. Biol. Chem. 38 (1919), 465–86.

[2] Bachmann, F. M. Vitamine Requirements of certain yeasts. J. Biol. Chem. 39, 235–58.

[3] Wildier, E. Nouvelle substance indespensible au developpement de la levure. La Cellule, 18 (1901) 313.

CHAPTER V

ORIGIN OF THE YEASTS, THEIR POSITION IN CLASSIFICATION OF THE FUNGI AND THEIR SYSTEMATIC RELATIONSHIPS

LET us now consider the morphological, cytological, and physiological characteristics of the yeasts. It is interesting to consider the place which they hold in the classification of the fungi. It has been stated, at the beginning of this book, that the sporangium of the yeasts is comparable to the asc in the *Ascomycetes*. It now remains for us to discuss the reasons for wishing to incorporate the yeasts under the *Ascomycetes*. Although this is definitely settled today, this question has been the object of such polemics that they are worthy of our attention.

(A) Historical

The question of the position of the yeasts in classification of the fungi has remained unsolved for quite a period of time. Do the yeasts make up an autonomous species or do they simply represent a stage in the development of the filamentous fungi, more advanced, which exist during the fruit season as yeasts, and during the winter as mycelial fungi? It is easy to observe the different stages in the life history of yeasts, the stages of budding and sporulation; but it has not been shown that the culture in artificial media presents the whole life cycle and that it may not be more complex in nature. Thus, we have seen in the early part of this book that many fungi present yeast-like structures during some stage in their life cycles. Such is the question that arose in the days when Pasteur worked and which ought to be answered in our day.

The subject is rendered more complex by the fact that little is known about their origin and life cycles. It is known that beer yeast has been handed down from brewery to brewery from time immemorial, and that other industrial yeasts used today may have their beginning in early Egyptian history. The domestic yeasts by continued cultivation by man may have been reduced to a constant form of a yeast. Such is not the case with wine yeasts. These exist naturally on the surface of the grapes and it is only necessary to press out the juice which will soon ferment. But where does this yeast come from which is on the surface of the grape?

Pasteur[1] was one of the first to attempt to answer this question. He began in 1875 a series of investigations to find out whether the yeasts could be isolated from the skin of the grapes and whether they were present only at one time of the year. At different times in the year he placed pieces of the vine and grape leaves in tubes of sterile wort. This experiment indicated that during the autumn the yeasts existed on practically all parts of the plant and that they were very unequally distributed on the grapes themselves. He further showed that the yeasts were present only during the period of maturity in the grape, and that it was not present at other times. The yeasts were found to be present during the fall, to gradually disappear during the winter.

Where do these yeasts come from? In what form do they pass the winter? The problem is an intricate one. Pasteur has remarked, however, that the yeast is always associated with another fungus, *Dematium pullulans* which, according to him, is present on the grape vine during the whole year. Pasteur thus thought that possibly this *Dematium pullulans* developed into the yeasts, and this theory seemed more plausible when it is remembered that this fungus has yeast-like stages in its life cycle. This idea of Pasteur's corroborated the assertions of the botanist Brefeld for whom the yeasts were only developmental forms for more complex fungi as the *Ustilagines*. Pasteur expressed it thus: "The yeast cells originate from little brown bodies (cysts of *Dematium*) which the microscope demonstrates so abundantly among the pollen of fruits." Pasteur soon gave up this idea, especially when the celebrated Chamberland showed that these yeasts of *Dematium* did not produce alcoholic fermentation.

This opinion has been especially maintained by Jörgensen.[2] According to this author the yeasts spring from yeast-like structures of *Dematium pullulans* as was thought by Pasteur. These were regarded as being constantly present in the atmosphere, and on the parts of grape vines, etc. These are supposed not to develope into the true *Saccharomyces* capable of producing alcohol and forming endospores.

Somewhat the same idea is expressed by Jühler who observed a fermentation in a flask of rice starch inoculated with *Aspergillus oryzae* which serves the Japanese in making saké. Jörgensen, also, believed a relation between the conidia of this fungus and the true yeast. This assertion has been sustained by Sorel.[3]

[1] Pasteur. Étude sur la bière, 1876.

[2] Jörgensen, A. Der Ursprung der Hefen. Cent. Bakt. 2, 1895. Ueber Ursprung der Alkoholhefen. Ber. d. Gärungsphysiol. Lab. von Jörgensen. Copenhagen, 1, 1895.

[3] Sorel. Étude sur *l'Aspergillus oryzae*. Comp. Rend. Acad. Sciences, 121, 1895.

The investigations of Seiter [1] and especially those of Hansen, Klöcker [2] and Schiönning have established with a remarkable precision that these investigators were led astray by impurities in their cultures. They have shown that the yeast-like structures of *Dematium* never sporulate, and that the leavening agent of saké consists, besides *Aspergillus oryzae*, of a yeast in no way related to the mold which induces the fermentation. The same authors have made repeated experiments to change yeasts into molds and molds into yeasts under conditions such as those which obtain in nature. They have never secured reliable results.

However the question of the origin of the yeasts from molds has been raised anew by the investigations of Viala and Pacottet [3] on *Gloeosporium ampelophagum* and *Gloeosporium nervisequum*. One of these fungi, *Gl. nervisequum* presents perithecia which have been observed by Klebahn who has classed it among the *Ascomycetes sphericeae*. The other, the *Gl. ampelophagum*, on account of the presence of pyknides, organs characteristic for the *Spheriaceae*, has also been placed in the same family of ascomycetes although perithecia have never been observed. According to Viala and Pacottet these two microörganisms are able to develop, when placed in suitable nutrient media, into true yeasts, capable of setting up the alcoholic fermentation and forming endospores identical in all respects with those formed by *Saccharomycetes*. These yeasts become fixed after cultivation in the same medium, and find it impossible to return to the mycelium state. Viala and Pacottet conclude that yeasts originate at the expense of more highly developed fungi and think that, if it is impossible to change the industrial yeasts into mycelial conditions, it is due to a long existence in the state of yeasts and it has become impossible to change. Thus according to these investigators the yeast sporangium may not possess the value of the asc but the endospores may result from an encystment of protoplasm without other morphological significance.

Guilliermond [4] in studying this question and trying to reproduce the results of Viala and Pacottet established that impurities in cul-

[1] Seiter, O. Studien ü. d. Abstammung d. Saccharomyceten. Cent. f. Bakt. 2, 1906.

[2] Klöcker, A., and Schiönning, H. Que savons-nous sur l'origine des Saccharomyceten. C. R. du Lab. de Carlsberg, 55, 1896.

[3] Viala, P. and Pacottet, P. Nouvelles recherches sur l'Anthracnose. Rev. de viticulture, 1905. Levures et kystes des Gloeosporium. Ann. Inst. Agr. 5, 1906.

[4] Guilliermond, A. A propos de l'origine des levures. Ann. mycologici, 5, 1907; Recherches sur le dev. du *Gl. nervisequum* et sa pretendue transformation en levures. Rev. gen. de Botanique, 20, 1908.

tures could explain the results of these two workers. In repeating the investigation on *Gl. nervisequum* he showed that this fungus never produced the yeast-like structures when grown in certain media. On the other hand, he established that the yeasts which these workers thought they observed as derivatives of *Gl. nervisequum* present the same characteristics as the yeast-like structures of a species of *Dematium* which he observed in the earlier inoculations of the *Gloeosporium*. This *Dematium* exists on all of the leaves of the plane tree and develop, almost always, in a state of impurity in the first artificial cultures of *Gl. nervisequum*. Then, to such data, Guilliermond attributes the conclusions of Viala and Pacottet. As for the endospores described by Viala and Pacottet in the yeast structures, they may have been simply fat droplets from old cells of *Dematium* which by the size and regular positions resemble the endospores of yeasts. Whatever is the case, it is definitely established that *Gl. nervisequum* does not form yeasts

(B) Studies in Life Cycles of Yeasts in Nature

This conclusion on the transformation of yeasts is fully confirmed by the careful investigations of Hansen [1] on the life cycles of yeasts. The first observations of this author date back to 1881, and are concerned with *Saccharomyces apiculatus*. This yeast is particularly adapted to life history studies on account of the special form of its cells. (Fig. 6.) Hansen observed that this yeast existed on many different fruits and that it was found only on the walls. It was only present on the fruits and not on other parts of the plant. It appears, then, that it lived only where there was sugar or where it was able to multiply.

Hansen thought that the rain and decay of the plant carried this to the ground on which fruit trees grow. It seems, then, that this yeast is able to hibernate in soil near fruit trees. If samples of this earth are taken in the springtime, *S. apiculatus* is always found. Finally to prove this hypothesis, he inoculated soil and left it out through the winter. From time to time, he sampled this soil and always found *Saccharomyces apiculatus*. Hansen has thus demonstrated that *Saccharomyces apiculatus* is able to perpetuate itself in the soil from year to year.

[1] Hansen, E. Recherches sur la physiologie et la morphologie des ferments alcooliques. Sur le *S. apiculatus* et sa circulation dans la nature. Comp. Rend. du lab. de Carlsberg, 1, Livr. 4. 1881. Nouvelles recherches sur la circulation du *S apiculatus* dans la nature. Ann. des. Sc. nat. et botanique, 7th Series, 1890.

On the other hand, he has shown that it passes the winter in the soil, for he examined other substances such as dust, dried fruit, animal excrement and never found this yeast.

The investigations of Müller-Thurgau[1] and Berlese[2] indicate somewhat the same things. Berlese has found, in April and June, *Saccharomyces apiculatus, ellipsoideus* and *Pastorianus* in the earth of vineyards and orchards. These yeasts were found down to 12 or 13 centimeters in depth and seemed to be equally distributed in both sunny and shady places. This is interesting for it shows the resistance of these yeasts to sun and light. Berlese has also found *S. apiculatus* on the bark of oak and olive trees, and also in the nectar of flowers.

Hansen[3] has undertaken, in recent years, a study of the life cycle of yeasts in nature to find out whether all of the yeasts behave like *S. apiculatus*. He used various yeasts in this investigation and experienced some difficulty, for the shapes of the various yeasts did not lend themselves to a ready recognition. They were very easy to confuse with the yeast forms of *Dematium* and other fungi. Only one character was available and that was the formation of endospores.

In his recent investigations, Hansen investigated the presence of yeasts in the soil about Copenhagen and whether they were present at all periods of the year. These environs included many orchards, gardens and vineyards. He was scarcely able to find a spot which did not contain yeast. They were almost always present in the surface layers and scarcely at all in the deeper layers; at all times of the year he was able to isolate them. The soil in vineyards and orchards was plentifully supplied with them but they diminished in numbers as one went away from the orchards. Thus, in 100 analyses of soil under fruit trees, 67 showed the presence of yeasts; away from such places in fields, only 19 per cent of the samples indicated the presence of yeasts.

Hansen has also observed yeasts in the soils of beech, fir, pine and oak groves but much less numerous than in fruit groves. Only 30 per cent of the samples yielded the presence of yeasts. Such yeasts belonged to special genera such as *Pichia membranefaciens* and *Willia anomala.*

[1] Müller-Thurgau, H. Ueber den Ursprung der Weinhefe. Weinbau Weinh. No. 40 and 41, 1889.

[2] Berlese, A. Verhalten des Saccharomyceten an den Weinstöcken über die Verteilung der alkoholischen Fermenten in der Nature. Ueber die Transportmittel der alkoholischen Fermente. Rivista di patol. vegetale 5, 1897.

[3] Hansen, E. C. Neue Untersuchungen über den Kreislauf der Hefenarten in der Natur. Cent. Bakt. 10, 1903. Ueber die Brutstätten der Alkolholgärungspilze oberhalf der Erde. Cent. Bakt. 14, 1905.

These investigations indicate that all of the yeasts studied by Hansen have a life history identical with that of *S. apiculatus*. The yeasts hibernate in the soil. They seem to differ only in their distribution. Hansen explained this on the basis of spore formation, assuming that yeast which formed no spores would be killed. On the other hand, thanks to the presence of spores, the yeasts live a longer time than *S. apiculatus* in the ground water which carries them for longer or shorter distances.

It is then necessary to determine the method by which the yeasts are transferred from the soil to the fruit skins. Transportation through the air seems to play an important rôle. Chamberland has observed that there are many yeasts in the air especially during summer and autumn. One may detect them at the other seasons but they are not so common. From this the yeasts seem to be less abundant during the rainy seasons of the year. Hansen [1] states that yeasts are always found in the atmosphere but in different numbers. Their number seems to be increased during June to August and especially at the beginning of September. During the other seasons, one may not find them as readily. Berlese did not find any yeasts in the air during April and May but was able to find *S. apiculatus* in the beginning of June and during July. Thus it seems that the air may be an important factor in transporting the yeasts from the ground to the fruit. On this, they find a higher temperature and more favorable environment and develop to maturity. The presence of yeasts, then, in the air seems to be a function of two factors: first, an active development of these organisms on the skin of the fruit and, secondly, an absence of rain.

Boutroux [2] has shown that insects play an important rôle in the distribution of yeasts. He disclosed the presence of yeasts on various insects (mosquitoes, wasps, bees, gnats and ants). *Saccharomyces cerevisiae, ellipsoideus* and *Pastorianus* were demonstrated. Wortmann and Berlese have observed the same things and regard the insects as the important mode of distribution of yeasts from grape to grape and from vine to vine. In this way, Berlese explains the presence of *S. apiculatus* in the nectar of flowers which has been visited by *Vespa crabro* in which he has observed the same yeast. He does not regard the deposition of the yeasts by the insects' feet with much favor but points out that the yeasts are able to pass through the intesti-

[1] Hansen, E. C. Recherches sur les organismes qui a différentes époques de l'annee, se trouvent dans l'air à Carlsberg et aux alentoirs. Comp. Rend. du lab. de Carlsberg, 1, 1882.

[2] Boutroux, L. Sur l'habitat et la conservation des levures apontanees. Bull. de la Soc. Linn. de Normandie, 3rd Series, 7, 1883; Ann. des Sc. nat. Bot. 17, 1884.

nal canal without harm. The intestinal canals of certain diptera seem to be the normal habitat for certain yeasts; in fact, he has observed *S. apiculatus* and *ellipsoideus*. Such conclusions are in accord with the work of Neumayer, who has demonstrated that yeasts are very resistant to digestive juices. It is well to point out that this means of dissemination is not mentioned by other authors, Hansen, for instance.

(C) Morphological and Cytological Investigations on Yeasts

It has just been stated that, under no circumstances, are we able to transform yeasts into molds, or a mold into a true yeast; this has not been observed in nature. Hansen did not hold this view and regarded the yeasts as an autonomous group of fungi, *Ascomycetes*.

Such an hypothesis was not a new one. Before this, Reess and de Bary had suggested this idea and noticed the superficial similarity between the asc of the yeasts and the sporangium of the molds. The asc is a single character which distinguishes between the true yeasts and yeast-like structures of other fungi. So little was known, then, about the cytological characteristics of the asc that it was difficult to make any definite statements.

For a long time this morphological problem remained untouched. Were the sporangia of yeasts similar to the ascs of the *Ascomycetes* as was maintained by Hansen? Or, should we regard them as approaching more closely the sporangia of the Mucors, as was thought by Brefeld? Do the yeasts represent a bona-fide group of fungi or are they developmental forms of the molds? These questions remained unanswered. One may always suppose that the yeasts resulted from the molds by some process, hitherto unobserved, and that they have lost the possibility of returning to the state of a mycelium. We have negative proofs in favor of the autonomy of the yeasts.

But in these later times, new facts have been discovered. It has been shown in the preceding chapter that the cytological studies on the asc and the discovery of sexuality in yeasts have furnished definite proof of the ascogenous nature of the sporangia of yeast, and have proven the relationship of the fungi to the *Ascomycetes*.[1]

[1] It might be well to point out that what distinguishes the group of Ascomycetes is their possession of an asc enclosing from 4 to 8 ascospores. The ascospores are differentiated on the interior of the asc only at the expense of part of the protoplasm. The rest, or epiplasm, is absorbed by the ascospores when they develop. Among the lower ascomycetes (Endomycetes) the ascs form only at the expense of terminal cells on the filaments. Among the higher Ascomycetes, they are united in great numbers in organs called perethecia.

A sexual process, rudimentary in certain types, always intervenes in the origin of the yeasts. Among the Exoascee there is a simple nuclear fusion. Among the

The investigations of Guilliermond [1] have indicated that by the morphological and cytological characteristics, the sporangium of the yeasts presents a remarkable similarity to the asci of the *Ascomycetes*. The ascospores develop by the same process.

The ascospores in certain yeasts present, on the other hand, characteristic forms absolutely analogous to the ascospores of certain *Ascomycetes*. Thus it is that the ascospores of *Willia anomala* are identical with those of *Endomyces decipiens*, *Endomyces fibuliger* and *Ascoidea rubescens*. Those of *Willia saturnus*, *Schwanniomyces occidentalis*, *Debaromyces globosus*, *Monospora cuspidata*, *Nematospora coryli* have forms which suggest very strongly those of certain ascomycetes. Without doubt, the number of ascospores in the sporangium of a yeast is variable although it is constant for an asc. However, one notices that the number of ascospores tends to become fixed in an asc in most of the yeasts while with some, it remains variable. Thus it is that in *Schizosaccharomyces* the number 4 or 8 is usually seen. In *Saccharomyces Ludwigii* the ascospores are constantly present to the number of 4. Even in those cases in which this varies, there is a slight tendency for it to become fixed.

Finally, the discovery of a copulation in the origin of the asc in *Schizosaccharomyces*, the *Lygosaccharomyces* and *Debaromyces globosus* which absolutely resembles that of certain Ascomycetes (*Bremascus* and *End. Magnusii*) furnishes a strong argument in favor of their homologation. The existence of this copulation, together with morphological and cytological characteristics of asci of yeasts, suffices to demonstrate their place with *Ascomycetes*. The question of the origin and systematic relationship of the yeasts is definitely settled today. The *Saccharomycetes* constitute an autonomous group of lower *Ascomycetes*. It has been stated that among the true yeasts which form asci, there are some which do not sporulate; such are the *Mycoderma* and *Torula*. But, as will be pointed out further on, many of the yeasts are able on account of special conditions, to definitely lose their property of sporulating. It is possible that these are true *Saccharomycetes* having become asporogenous but it is also possible that they are derived forms from molds fixed in the state of yeasts. The question of their origin and their position in classificatory systems is then not settled. Ought we to separate the family of *Saccharomyces* in which are the true yeasts?

higher Ascomycetes, less is known. According to Harper, it consists of a true copulation to give the perethecia; according to Dangeard, it is simply a nuclear fusion. The question is still very obscure. Among the Endomyces, copulation is very clear.

[1] Guilliermond, A. L'origine des levures. Annales mycologici, 5, 1907.

Phylogeny of the Yeasts. Their Affinities in the Group of Ascomycetes

What place, in the classification of the *Ascomycetes*, shall the yeasts occupy, what are their relationships to the other *Ascomycetes?* We shall now take up that question. Up to recent times, it seemed incapable of being answered.

The species of *Exoascus* are filamentous fungi, in which certain terminal cells form octosporous ascs after a fashion comparable to those formed by the yeasts. The ascospores germinate, producing generations of yeasts. It is evident that, by the characters of their ascs and the shape of the yeast-like structures to which they give birth, they are like the *Saccharomyces*. They differ in the presence of a typical mycelium. But we have seen that the yeasts themselves, under certain conditions are able to manifest a tendency, more or less marked, to vegetate with a mycelium. The investigations of Dangeard and Ikeno have shown that the asc in *Exoascus* possesses two nuclei at the time of its formation, and these fuse into one having the nuclear divisions necessitated by 8 ascospores. Dangeard regards this fusion as karyogamy and the equivalent of fecundation but the interpretation of this process remains very much discussed. The yeasts are closely distinguished from *Exoascus* in that they show no nuclear fusion in the asc. It is true that in a few varieties, the asc results from a true copulation but in all of the varieties in which this phenomenon is lacking one is unable to detect nuclear fusion. Then, from this point of view, the yeasts resemble *Exoascus*.

On the other hand it has been noticed for a long time that the family of *Endomyces* includes varieties related to the yeasts. But our information with regard to this group has remained very vague.

Recent investigations by Guilliermond [1] have allowed us to fill this gap in our knowledge and at the same time determine the systematic relationships of the yeasts. The results of these investigations are sufficiently important to receive more extended treatment at this time. The family of *Endomycetes* presents only a small number of representatives in which the genera *Eremascus* and *Endomyces* are best known. We shall take up some of the shapes of these genera.

Only two genera of *Eremascus*, the *E. albus* and *E. fertilis*, recently discovered by Stoppel, have been known up until recently. The former is not well known; the latter has been subjected to a conscientious investigation by Stoppel whose results were confirmed by Guilliermond. The mycelium of *E. fertilis* presents cells which

[1] Guilliermond, A. Recherches cytologiques et taxonomiques sur les Endomycétées. Rev. g. de Botan. 26, 1909.

are generally mononuclear. It never produces conidia but, on the contrary, forms a rather large number of ascs. These are derived from an isogamic copulation which is accomplished, usually, between two contiguous cells in the same filament. The two cells unite by means of little canals playing the rôle of gametes, which anastomose, forming in this way, a sort of bridge between the two cells. (Fig. 54.) The wall which separates the two cells at the middle of the copulation canal is not slow to break down. Part of the cytoplasm enters the canal from each cell and forms a swelling at the middle of the copulation canal which becomes the zygospore. At this moment each of the cells divides its nucleus. One of the daughter nuclei thus formed remains in the cell and the other passes into the zygospore. (Fig. 54.) There the two sexual nuclei fuse and develop into a single large one. As this proceeds the zygospore forms a wall which separates it from the two threads which formed it. From this the zygospore grows and develops into an octosporous asc quite similar to that of a yeast. The ascospores are enveloped as those of *Saccharomyces guttulatus* by a double membrane in which the external one breaks at the moment of germination. They germinate directly into a mycelium. It cannot be refuted that *Eremascus* resembles the yeasts; its ascs present the same characteristics as those of the yeasts and result from a copulation which is able to be approached by cells which one sees in the *Zygosaccharomyces* and *Schizosaccharomyces*. By the copulation which precedes the formation of the ascs, many yeasts are similar. In most of the yeasts, it is true, copulation differs from that of *E. fertilis* in that it is incomplete and ends in the formation of an asc having the form of a dumb-bell; *Schizosaccharomyces octosporus* offers an intermediate stage between the copulation of *Eremascus* and that of the yeasts. In this yeast, copulation is more often complete and produces a large oval cell which is transformed into an asc. In this case, copulation is absolutely homologous to that of *E. fertilis*. In reality, *E. fertilis* differs especially from the

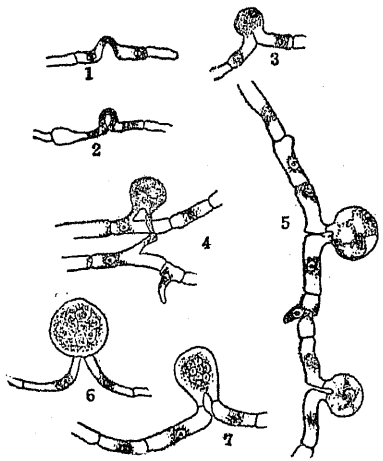


Fig. 54. — Different Stages in the Copulation and Formation of Ascs in *Eremascus fertilis;* 1 and 2: Beginning of Copulation between Two Contiguous Cells; 3 and 4: Copulation; 5: Demarcation of the Asc; 6 and 7: Formation of the Ascospores.

yeasts in that yeasts are reduced to the state of isolated cells while the *E. fertilis* remains in a mycelial condition.

With the genus *Endomyces*, one begins to approach the yeasts. *Endomyces fibuliger*, discovered by Lindner, shows striking resemblances to *Eremascus fertilis*. It differs, however, by that fact that the mycelium formed from uninuclear cells gives birth, by budding, to a series of yeast-like structures (Fig. 55) which suggests that this fungus is intermediary between the yeasts and *Eremascus*. Under certain conditions, it is able to vegetate exclusively with the form of yeasts. *E. fibuliger*, on the other hand, produces conidia which form themselves by budding and are able to be compared, to a certain extent, with the "durable cells" of yeast. Finally, it furnished numerous ascs very similar to those of *Eremascus* which contain only 4 ascospores. These ascs are formed often simply by budding of the elements, but in many asces, they form after attempts at copulation at the expense of an anastomosis which occurs between two neighboring cells taking place in the following manner: Two units of the mycelium send out little rootlets. These anastomose but the wall which is formed between them does not break down and, in many cases, there is no mixture of the cell contents. Generally one of the protuberances stops developing, the other elongates, bends itself toward the first and forms by a swelling, a tetrasporous asc. (Figs. 56, 1, 2, 3, 5, and 6.) In the meantime the two rootlets develop into an asc. In some cases, the two protuberances progress side by side, without anastomosis, each forming a swelling which becomes the mother cell of an asc; these two cells, thus formed, bind themselves one to the other by a sort of copulation canal in which the wall is not broken down. It also happens that the extremities of a filament, formed by the walling off of a chain of cells which swell up, transforms itself into an asc. Often, in this case, anastomosis is often noticed binding the ascs two by two.

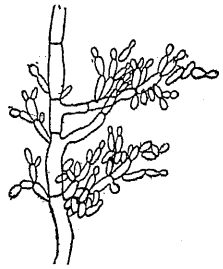


Fig. 55. — Mycelium in *Endomyces fibuliger* Forming a Number of Yeast-Like Bodies.

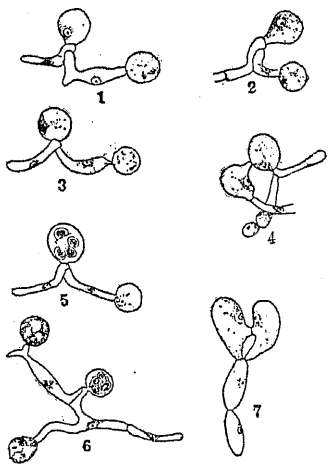


Fig. 56. — Different Stages in the Formation of Ascs in *Endomyces fibuliger*.

These anastomoses prove then, that, although sexuality may have disappeared, there seems to be a rudimentary sexual attraction quite comparable to the phenomena which have been observed in certain yeasts (*Schw. occidentalis*, yeasts of Rose and Dombrowski, etc.). However when one compares these anastomoses with the sexual production of *Eremascus fertilis*, he is struck by the resemblance which exists between the method of formation of ascs in these two fungi (Fig. 57). In one and the other, two contiguous cells produce protuberances which seem to search for each other. With *Eremascus fertilis*, they reunite to form an egg while in *E. fibuliger* they constantly fail in their attempt. (Fig. 57, A and B.) It is not doubtful that the anastomosis which precedes the formation of the asc in the latter fungus represents traces of an ancestral reproduction analogous to that which occurs in *Eremascus fertilis* to which *E. fibuliger* is closely related. We may then regard *E. fibuliger* as a form derived from a genus neighboring *Eremascus fertilis*. The ascospores have the same form as those of *Willia* anomala; they are hemispherical and provided with a projecting color giving them the appearance of a hat. On the other hand, they are supplied, like those of *E. fertilis*, with two membranes. The external membrane is burst during germination. The ascospores germinate either in the form of yeasts or with a mycelium.

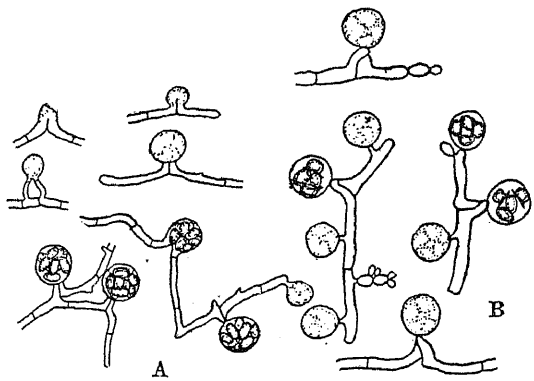


Fig. 57. — A. Showing Copulation and Asc Formation in Eremascus fertilis; B, The Same for *Endomyces fibuliger*.

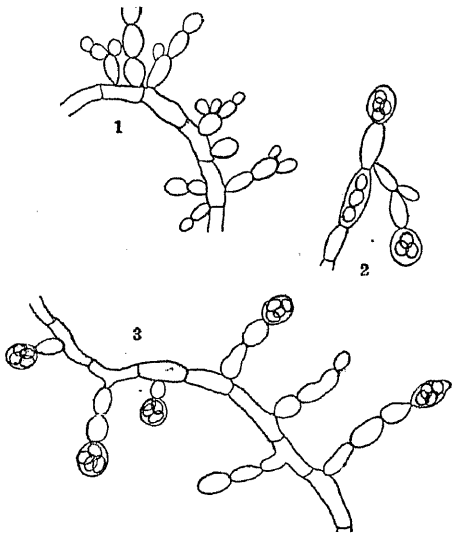


Fig. 58. — *Endomyces capsularis*.

1, Fragment of the Mycelium Showing the Formation of Yeasts. 2 and 3, Fragment of the mycelium Producing Ascs.

Endomyces fibuliger [1] constitutes a link between *Eremascus fertilis* and *Endomyces Hordei* recently described by Saito. This last-mentioned species has the same characteristics as *Endomyces fibuliger* with the exception that no conidia, but only yeast forms, are found. It forms ascospores in the shape of a hat but these ascs result from simple budding of the mycelium without presenting an anastomosis. All traces of sexuality have disappeared. The ascospores have a double membrane and germinate by simple budding. *Endomyces Hordei* represents a higher step in the parthenogenetic evolution than seems to have taken place in the descendants of *Eremascus*.

With *Endomyces capsularis*, discovered a few years ago by Schiönning [2] we have a similar species but one more closely connected to the yeasts. This fungus also has a branching mycelium with septa made of cells with one nucleus and which form numerous yeast bodies by budding. These, however, are much larger in number than in *Endomyces fibuliger* and *Endomyces Hordei*. *Endomyces capsularis* also has ascs with its ascospores possessing a double membrane and germinating either into yeast bodies or a mycelium. The ascs are formed as in *Endomyces Hordei* by a sort of budding of the cells or of any cell in the mycelium without any anastomosis.

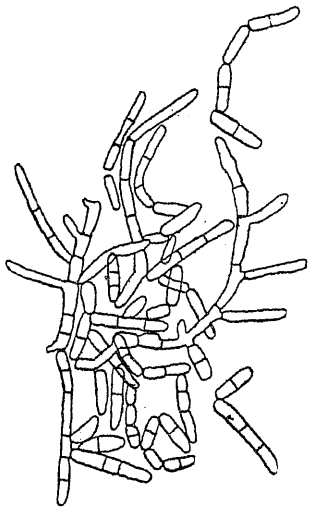


Fig. 59. — Fragment of Mycelium in *Endomyces Magnusii* in the Process of Breaking into Oidia (after Rose).

End. javanensis, described by Klöcker, [3] offers a form of transition more disputed between *Endomyces* and the yeasts. The mycelium is greatly reduced and yeast forms predominate. The ascs, always parthenogenetic, form indifferently at the expense of some cells in the mycelium or of a yeast cell. They include a single ascospore much like the ascospore of *Sch. occidentalis*. They germinate either into the form of yeasts or a mycelium. This fungus presents, then, such great resemblances to the yeasts that it is difficult to know whether it should be classed among the *Endomyces* or among the

[1] Lindner, P. Endomyces fibuliger, n. sp. einer neuer Gärungspilze und Erzeuger des sog. Kreidekrankheit des Brotes. Woch. Baruerei, 24, 1908.

[2] Schiönning. Nouveau genre de la famille des Saccharomycetees. Comp. Rend. trav. lab. Carlsberg, 6, 1908.

[3] Klöcker, A. Endomyces javanensis, nov. sp. Comp. Rend. des trav. du lab. de Carlsberg, 6, 1909.

yeasts. Nevertheless since the essential characteristic of the genus *Endomyces* is the presence of a typical mycelium from which the ascs spring exclusively, it seems that *E. javanensis* ought to be regarded as a yeast.

The information with regard to these various fungi explains anew the phylogeny of the yeasts. Indeed, it is possible to regard the genus *Eremascus* as an ancestral form. From this may originate a form, quite hypothetical, related to *Endomyces fibuliger*, but differing by the existence of an isogamic copulation characteristic of *Eremascus*. This copulation which is reduced to an unfruitful attempt with *E. fibuliger* has completely disappeared in *E. capsularis*. From this hypothetical form the yeasts may derive by regression and from the mycelial form which yields its place to yeast-like forms.

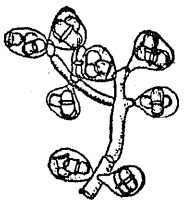


Fig. 60. — Ascs of *Endomyces decipiens* (after de Bary).

Summarizing, this hypothetical fungus, derived from Eremascus, may be the beginning of two branches, one with *E. fibuliger* and the *E. capsularis*, the other with *Zygosaccharomyces* and the *Saccharomyces*. The genus *Saccharomyces* represents a parthenogenetic form derived from *Zygosaccharomyces*.

Now it remains to determine the origin of the *Schizosaccharomyces*. The study of two other forms of the genus *Endomyces*, the *E. Magnusii* and the *E. decipiens*, has given some information on the subject.

These two fungi resemble very much, in the whole of their development, *E. fibuliger;* but they are closely distinguished by the fact that, in place of producing yeast-like bodies, they form, by dissociation of their mycelium, cells called oidia which are capable of dividing transversely like the cells of *Schizosaccharomyces*. (Fig 59.) Let us state that, in certain media, a true mycelium is not formed but almost always oidia which multiply like the Saccharomyces. In its general form, the oidium is identical to the cell of *Schizosaccharomyces*. Cytologically, however, it differs more often in *E. Magnusii* by the presence of many nuclei. Nevertheless, many of the oidia of *E. Magnusii* offer only a single nucleus and Dangeard has shown that in the oidia of *E. decipiens*, this is always the case.

These two fungi present also chlamodyspores which are formed like oidia by a sort of dissociation of cells in the mycelium but are distinguished by the formation of a very thick membrane and by the fact they cease to divide until they find conditions sufficiently favorable. These, then, are sort of encysted oidia and may be compared to the durable cells of yeasts.

Finally, *E. decipiens* and *E. Magnusii* produce numerous ascs which form at the extremities of the filaments. In *E. decipiens*, they are not preceded by a sexual act, but in *E. magnusii* a heterogamic copulation has been established which results in the asc. (Fig. 6.) This is accomplished between a male gamete and a female gamete, each being at the end of a filament. The male gamete is a small, short, cell, with a single nucleus which is located at the end with a shape like a screw. The female gamete is a long cell which also in-

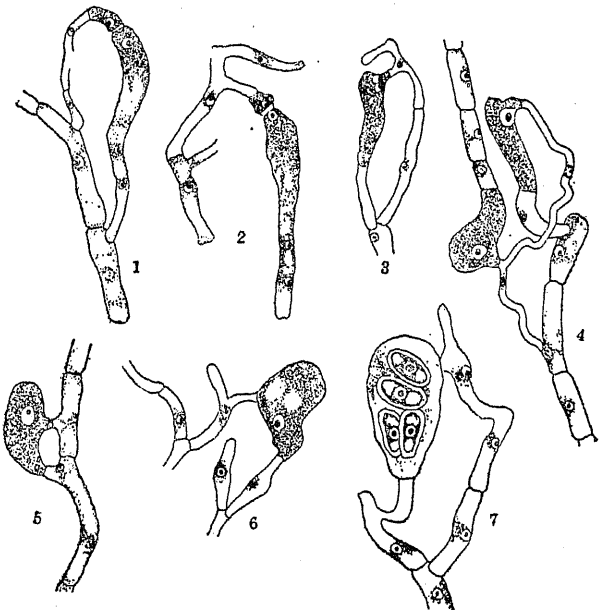


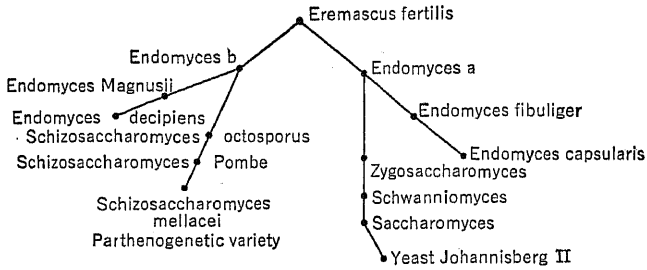
Fig. 61. — Various Stages in the Copulation and Formation of Ascs in *Endomyces Magnusii*.

cludes a single nucleus. The gametes unite by their ends. (Fig. 61, 1 and 2.) The middle wall which separates them breaks down, their contents fuse protoplasm with protoplasm, nucleus with nucleus. The egg thus formed grows and is transformed into a tetrasporous asc. Although, heterogamic, this copulation resembles very much that of *Sch. octosporous*.

It looks as if one might regard the *Schizosaccharomyces* as derived from a hypothetical form analogous to *E. Magnusii* but more advanced, in which the copulation may be isogamic. From this form comes on one side *E. Magnusii* and its parthenogenetic form, *E. decipiens*, and on the other part *Schizosaccharomyces*.

The scheme presented below represents the different steps in the phylogeny of the yeasts according to the theory which has been out-

lined. The budding yeasts are sprung from a hypothetical form, *Endomyces a*, analogous to *End. fibuliger* but have kept the copulation of *Eremascus*. This copulation persists in the *Zygosaccharomyces*, no trace of it remains in the *Schwanniomyces*, disappears completely in the *Saccharomyces* and is replaced by a parthenogamy between the ascospores in the yeast Johannisberg II. The *Schizosaccharomyces* spring from a hypothetical form, *Endomyces b*, related to *End. Magnussii* but with isogamic copulation. The *Schizosaccharomyces* seem, like other yeasts, to be more advanced toward parthenogenesis as is evidenced by a variety, *Sch. mellacei*, which has lost its sexuality.



Summarizing, it seems proper to consider the *Saccharomyces* and other budding yeasts and the *Schizosaccharomyces* as derived from a form related to *Eremascus fertilis*. From this common stock, two branches spring: one which forms the *E. Magnusii* and Schizosaccharomyces, the other which forms *E. fibuliger*, the *Zygosaccharomyces* and the budding yeasts. The question of the phylogeny of the yeasts may be considered today as a little more settled.[1]

[1] Another theory has been recently proposed by Nadson following his discovery of *Nadsonia fulvescens*. According to this author the *Endomycetaceae* and the *Saccharomycetaceae* represent degraded forms derived from the higher Ascomycetes. The yeasts possess a copulation in the germination of the ascospores with *Saccharomyces Ludwigii* being an archaic yeast. This theory lacks a solid foundation because it does not provide for any of the links between the higher ascomycetes and the yeasts. On the contrary, Guilliermond's theory rests on a series of known facts.

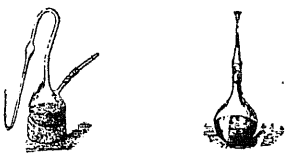
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CHAPTER VI

METHODS OF CULTURE AND ISOLATION OF YEASTS. PROCEDURES FOR THEIR STUDY

A. Methods of Culture

WITH the exception of a few pathogenic varieties, all of the yeasts isolated up to the present time, grow well on artificial media. They may be cultivated according to the same methods as bacteria. These procedures are sufficiently well known and are outlined in detail in all books on bacteriology. It would be out of place to mention them in a book of this nature. It will suffice to mention here a few of the media and methods which are especially adapted to the growth of yeasts. Like the bacteria the yeast may be cultivated as well on liquid as on solid media. As a rule, unlike the bacteria, the yeasts desire a slightly acid medium. The yeasts, although facultative anaerobic, multiply only on media which are well aerated. Since yeasts vegetate more often at the bottom of cultures, it is then necessary to place them in thin layers of medium in order to supply as much air as possible. On the contrary, if a fermentation is desired, they should be placed under conditions with a limited supply of oxygen and in a sugar medium contained in deep flasks or tubes.



A B

Fig. 62. — A, Pasteur Flask; B, Chamberland Flask.

For the culture of yeasts the same ordinary apparatus is utilized as in the study of bacteria (Petri dishes, Erlenmeyer flasks, Roux tubes, cover glasses, test tubes). For physiological investigations, Pasteur, Chamberland, Freudenreich and Hansen flasks are serviceable. The Pasteur flask is shown in Fig. 62. It is provided with two outlet tubes, one of which is bent and which contains a bit of cotton to filter the air which is thus able to pass in. The other is a long straight tube which enters the flask at the side. It is closed with a piece of rubber tubing carrying a piece of glass rod in one end. The other end of this tube is slipped over the tube from the flask. Sterilization may be accomplished by putting in boiling water. During this sterilization, the rubber tube may be removed from the

straight side arm. It may be replaced as soon as the operation is completed. It is not necessary to sterilize in the autoclave. The Chamberland flask (Fig. 62) is an ordinary flask in which the collar is drawn out and ground to receive tightly a straight cap which, in turn, is drawn out. A piece of cotton may be inserted in this. The Freudenreich flask is constructed a little after the same fashion but differs in that the body of the flask is cylindrical instead of spherical. These may be sterilized in the autoclave.

Some of the common media which may be used in the cultivation of the yeasts are mentioned below.[1]

Pasteur's medium:

Distilled water	1000	grams.
Candied sugar	20	"
Ammonium tartrate	0.1	"
or, Ammonium carbonate	1.0	"
Ash of yeasts	1.0	"

This medium was used by Pasteur in the greater part of his studies on alcoholic fermentation.

Hansen's Medium No. 1.

Peptone	1	gram
Maltose	5	"
Potassium phosphate	0.3	"
Magnesium sulfate	0.2	"
Distilled water	100.0	"

Hansen's Medium No. 2

Peptone	1.0	gram
Maltose	5.0	"
Potassium phosphate	0.3	"
Magnesium sulfate	0.5	"
Distilled water	100.0	"

Mayer's Culture Fluid:

Sugar	15	grams.
Potassium phosphate	5	"
Magnesium sulfate	5	"
Calcium phosphate	0.5	"
Ammonium nitrate	0.75	"
Distilled water	1000.00	c.c.

According to Mayer, this is a very useful medium for culturing yeasts.

[1] Investigations by Wildier in 1901, by Williams in 1919 and Bachmann in 1919 indicate that some vitamine-like substance may be necessary for the growth of yeasts. Apparently ordinary synthetic media alone are not entirely satisfactory for the culture of yeasts.

Laurent's Medium:

Ammonium sulfate	4.71	grams.
Potassium phosphate	0.75	"
Magnesium sulfate	0.1	"
Distilled water	1000.00	"

To this medium any sugar may be added. It was used by Laurent in his investigations on the hydrocarbon nutrition studies on yeasts.

Haydruck's Medium:

Water	2000	grams.
Saccharose	100	"
Asparagin	2.5	"
Potassium acid phosphate	50.00	"
Magnesium sulfate	17.00	"

Cohn's Solution:

Distilled water	200	grams.
Ammonium tartrate	2	"
Potassium phosphate	2	"
Magnesium sulfate	1	"
Calcium phosphate (dibasic)	0.1	"
Sugar	20	"

Noegeli's Medium (No. 3).

Distilled water	100	grams.
Glucose	3	"
Ammonium tartrate	0.04	"
Magnesium sulfate	0.04	"
Calcium chloride	0.02	"

Raulin's Medium:

Distilled water	1500.00	grams.
Candied sugar	70.00	"
Ammonium nitrate	4.00	"
Tartaric acid	4.00	"
Potassium carbonate	0.60	"
Magnesium carbonate	0.60	"
Ammonium sulfate	0.25	"
Ferric sulfate	0.07	"
Zinc sulfate	0.07	"
Potassium sulfate	0.07	"
Potassium silicate	0.07	"

This liquid, composed by Raulin in the course of investigations on the nutrition of *Sterigmatocystis nigra*, makes up a medium which is well adapted to the develpoment of fungi. It serves also for the growth of many fungi and molds. For the yeasts, however, it does not lend itself as well since they seem unable to grow in it. For a certain few special yeasts it will work.

Yeasts are easily cultivated on decoctions of various fruits. Malt extracts and fruit juices, such as prune juice together with decoctions of carrots, potatoes, etc., make good media. Beer wort is the best medium for the yeasts and the one which is most utilized for their culture. It may be procured at breweries or prepared in the laboratory after the following procedure: Soak 200 grams of malt, which has been previously pounded, in a liter of cold water and bring slowly to a temperature of 60° C. Shake once in a while, and after three-quarters of an hour, add 4 grams of hops. Boil for about an hour and filter. Test the filtrate for maltose by means of Fehling's solutions. The filtrate is then diluted with distilled water to yield a 3 per cent solution of maltose. The wort is then filtered and sterilized at 115° for 20 minutes.

The malt water is prepared by soaking 100 grams of germinated barley, which has been previously boiled, in a liter of water. This is then heated to 55–58° C. so that the amylase is not destroyed. Finally it is boiled for 5 minutes and filtered for sterilization.

Raisin extract is easily prepared by soaking a few grams of raisins in a little water and filtering. The filtrate may be sterilized at 150° for 20 minutes.

Another very useful medium is yeast water prepared as follows: 100 grams of fresh yeast are boiled, with shaking, in a liter of distilled water. This is filtered and sterilized. Yeast water is made up of ammonium salts, as paragin and peptones, which are ideal substances for yeast growth. This liquid by itself is generally insufficient. In order to secure abundant yeast growth, it is necessary to add a sugar. The decoctions of meat and various peptone media are used for some of the pathogenic yeasts.

Yeasts grow equally well on solid media. It serves because they sporulate easily in it and because they exhibit certain macroscopic characteristics which are used in their determination. Slants of potato, beet and especially carrot and even sterilized fruit juices are excellent media. These may be solidified in media. Beer wort may also be used as a solid medium. It is sufficient to mix 8 per cent of gelatin with it.

Methods for Obtaining Sporulation

The study of sporulation among yeasts required a special technique which might be outlined at this time. Special conditions have been mentioned above. The cells should be well nourished and young. It is necessary that they have acquired a sufficient reserve in their protoplasm to assure the formation of ascospores. It is necessary, then, to cultivate the yeast which one wishes to study, in a nutrient

medium for about 48 hours with frequent transfers. For this, beer wort is generally used. It may be necessary for the rejuvenating medium to contain some special substance which will stimulate the formation of ascospores.

The best method by which to make the yeast sporulate is to subject it to a period of inanition. Under such conditions the yeast, finding it impossible to vegetate, forms ascospores. The method devised by Engel and perfected by Hansen is most satisfactory; it consists of placing a block of plaster of Paris in the beer wort. This plaster of Paris is mixed with three parts of water and molded into a cylinder or truncated cone. It is important that the surface be smooth.

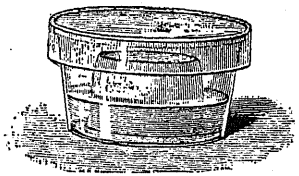


Fig. 63. — Crystallizing Dish Used for Sporulation.

The conditions for sporulation have been mentioned in a former chapter. It is known that certain factors are indispensable: the free access of air, favorable temperature, a certain degree of humidity, a medium which is not too acid, not too alkaline with favorable concentration. In order to realize these conditions, the block of plaster of Paris is placed in a dish in the bottom of which is a little distilled water. (Fig. 63.) Enough water ought to be in the dish so that about half of the block is covered. The water ought never to cover the block, for the block will absorb sufficient to support the development of the yeast which is placed upon it. In this way the yeast will find just those essentials which are necessary for its growth. The dish is closed by a cover in such a way that there is full circulation of air. The apparatus, thus prepared, is sterilized in the autoclave at 115° C. for a half hour.



Fig. 64. — Hansen's Flask for Culturing Yeasts.

The rejuvenated yeast is placed on the block of plaster of Paris. This operation is a very delicate one. If the yeast has been cultured in a liquid medium, the cells may be filtered out. By means of a sterile platinum wire some of the cells are then transferred to the surface of the block. If the culture of yeast is in gelatin, it may be transferred directly to the block. The dish is then covered and put in the incubator at a temperature depending on the yeast under examination. It has been stated that each yeast has an optimum temperature at which it sporulates, which is generally between 25° and 30°. At the end of thirty hours most of the cells will have sporulated.

To avoid the easy infection of the dish by bacteria, Hansen has devised a special flask which has received the name of the "Hansen

flask." (Fig. 64.) It is a cylindrical flask fitted with a glass top ground on with emery. This is pulled out and plugged with a bit of cotton. A side tube is closed with a piece of rubber tubing. A cylindrical block of plaster is put into the flask about which is placed bouillon. The apparatus is sterilized at 115° in the autoclave. The yeast is introduced into the flask in the usual manner.

Gorodkowa [1] has recently proposed a new method which has the advantage of being less complicated than the method of Engel-Hansen. It seems to give equally good results. It consists simply in inoculating a gelatin mixture with cells of the active young yeast. This medium is prepared as follows:

Distilled water	100	grams.
Gelatin	1	"
Meat bouillon	1	"
Sodium chloride	0.5	"
Glucose	0.25	"

The yeast develops quite rapidly after inoculation and the small amount of glucose is not sufficient to assure its nutrition for a long time. Thus, sporulation is stopped at the end of two or three days. This medium was utilized by Guilliermond for many yeasts with quite gratifying results.

Many other methods, founded on somewhat the same principles, have been perfected to demonstrate ascospores. Some consist of putting the yeast on blotting paper in distilled water or on pure gelatin. (Wasserzug.) Yeast water will also allow ascospore formation which is not sufficient to assure the nutrition of the yeast. It soon finds itself reduced to a condition which makes it sporulate. All sorts of liquids placed in extremely thin layers are sufficient to make a yeast sporulate if the food is quickly exhausted.

Yeasts will also sporulate on solid media (nutrient agar or gelatin). Rees has shown that slices of carrot make a good substrate for this purpose. The great majority of yeasts form ascopores after from 6 to 8 days, sometimes before. They grow actively for a few days and then budding slows up probably on account of an accumulation of toxic substances in the medium and sporulation begins. This method is to be recommended for cytological investigations, for it permits observations from the germination of an ascospore to the formation of a new ascospore. It facilitates the fixations which are necessary by making it easy to cut out a piece of the carrot on which the yeast is growing and placing it in the fixing bath. Guilliermond used this procedure with success in his investigations on the copula-

[1] Gorodkowa. Ueber das Verfahren rasch die Sporen von Hefepilzen zugewinnen. Bull. Jard. Bot., Petersburg, Vol. 1, 1908.

tion and sporulation of yeasts. It has the disadvantage of taking quite a little time with some yeasts.

Certain varieties sporulate quickly in special media. Thus, Klöcker has shown that *Schwanniomyces occidentalis* produces an abundant spore formation on a decoction of hay with gelatin. On the other hand, *Saccharomyces Ludwigii* sporulates in a short time in a 5 per cent solution of saccharose which is a poor nutrient for this yeast.

Other yeasts form ascospores in many common liquids when foods become scarce. In this category belong the varieties of the genera *Willia* and *Pichia*, which sporulate ordinarily in the pellicle on the surface of the media. The *Sch. octosporus* is able, oftentimes, to form ascospores at the beginning of fermentation.

These are the principal methods which are employed for this purpose. The method wherein the plaster block is used is satisfactory for most yeasts and is very convenient. Certain yeasts, however, furnish few or no spores with this method. The *Schizosaccharomyces* sporulate very easily on solid media (gelatin and carrot); the *Zyg. japonicus* shows spores only on nutrient gelatin, on Gorodkowa's medium and the pellicles of cultures. *Zyg. Priorianus* sporulates on a plaster block soaked with beer wort, on carrot and on Gorodkowa's medium. Finally certain parasites demand very special conditions. *S. guttulatus*, for example, sporulates only in the intestines of animals in which it lives as a parasite. This, however, is an exceptional case. *Zyg. major* sporulates only on plaster blocks which have been moistened with beer wort and on gelatin to which milk has been added.

In summarizing this question, it is well to note that the method of Engel-Hansen is the most widely used and after that the procedure of Gorodkowa. These yield most consistent results to which every one has recourse for careful studies on sporulation.

B. Methods for Purification and Isolation of Yeasts

The purification and isolation of yeasts require delicate technique. In nature, it happens that yeasts are not only mixed with bacteria and other fungi but also with other yeasts. It is quite simple to separate the bacteria and molds but more difficult to separate the yeasts from one another. The yeasts may possess the same shape, which makes it difficult to distinguish between them. Thanks to the careful investigations of Hansen and Lindner, we possess today such methods that the isolation may be made with fair certainty. Two methods are available, the physiological and the dilution method. The latter may be regarded as fractional culturing.

Physiological method: This procedure rests on the fact that the organisms in a mixed culture multiply unequally in the given medium and at the given temperature. Certain species die or vegetate slowly; they are finally eliminated by the most vigorous varieties. This is, then, a selection by vital concurrence which results in the elimination of certain species by others. Thus, to separate bacteria from a yeast a small quantity of acid is added to the culture medium (tartaric, lactic, hydrochloric or hydrofluoric). The bacteria prefer the alkaline media, while the yeast finds the acid most favorable. The yeast alone develops in this medium. If, on the contrary, one wishes to secure the bacteria a little alkali is added to the medium. By cultivating a mixture of yeasts in chemically different media and at different temperatures, one is able to separate them. Some will find one medium and temperature more favorable.

This method, which has been employed by many of the early workers, particularly by Pasteur and Cohn, is purely empirical and does not give reliable results. One may never exactly know what to expect with it. It is easy to suppose, for example, that one variety may be temporarily eliminated for the time being by the development of another form which finds the conditions more favorable; this last variety, however, after developing rapidly, may finally be suppressed by another form which has been dormant up to this time. Many varieties may develop together if all of the conditions are favorable. This was the case when Pasteur purified brewery yeast by this method. He cultivated his yeast in a solution containing a little sugar to which was added a small amount of tartaric acid. Later investigations by Hansen showed that Pasteur had eliminated the bacteria associated with the yeasts, but he had failed to effect a separation of the different varieties of wild yeasts, some of which caused the diseases of beer.

It is, then, impossible to secure reliable results by the physiological method of dilution. It is valuable, however, in starting the purification because it allows the bacteria to be separated from the yeasts. The yeasts may be only definitely separated by the procedure which we shall now take up.

Dilution Method for Separating Yeasts: This involves a mixture of the microörganisms which one wishes to separate until the cells are well isolated.

Lister conceived this procedure for the separation of lactic acid bacteria. He counted the number of bacteria in a drop of sour milk under the microscope and from this computed the required amount of distilled water which it would be necessary to add to this drop so that a drop of the mixture would contain a single cell. He prepared such a

dilution and added five drops to five flasks of sterile milk. Some of the flasks remained sterile while others seemed to possess a pure culture of lactic acid bacteria. These probably came from a single cell. Pasteur made the first application of this method to the purification of yeasts. He dried a small amount of yeast and after reducing it to a powder mixed it with plaster of Paris. He allowed this mixture to fall from a great height to make a dust. At this moment, he opened flasks of sterile media. Some of the dust particles carrying yeast cells fell into the flasks and in this way gave him pure cultures.

The dilution method is infinitely more certain than the physiological method. However, it does not yield absolutely sure results but only probabilities. How is it possible to affirm that a pure culture secured by this method came from a single cell? In spite of its fundamental imperfection, however, Hansen has devised two steps founded on the same principle, which allows the necessary accuracy.

These were perfected in 1881 by Hansen.[1] Part of the yeast culture is placed in a flask and is diluted with distilled (sterile) water. After shaking this flask the cells will be separated uniformly in the water. A drop of this liquid is taken and the cells counted under the microscope. A drop may be placed under a cover glass for examination. Let us suppose that there are 10 cells in the drop. If such a drop is put into a flask and diluted with 20 c.c. of sterile water, each cell should be separated after shaking. If one cubic centimeter of this liquid should be put into twenty sterile flasks, theoretically one-half of the flasks should show yeast growth. Practically, the results are somewhat different, for it is improbable that all of the flasks received a single cell. Hansen overcame this difficulty by shaking the flasks vigorously to separate the cells and to distribute them equally in all of the dilution water. The flasks were allowed to remain quiet until the yeast had developed into colonies. These could be seen on the bottom of the flasks. The number of colonies which developed gave some indication of the number of single cells which were present.

Hansen's second method consisted in the employment of a solid medium. Gelatin or agar was used. He secured his idea from Koch's work which today is always used. Koch's procedure involved the mixing of a part of the culture in a large amount of sterile water. A drop of this mixture was introduced into a flask of gelatin at 30°. This was well shaken to separate the microörganisms in the medium. This gelatin was then poured out onto plates of glass which were in-

[1] Hansen, E. C. Chambre humide pour la culture des org. microscop. Comp. Rend. lab. de Carlsberg, 3, 1881.

cubated under sterile covers. The gelatin solidified and the cells which were contained in it developed into colonies where they lodged. Macroscopic examination of the color of the colonies together with the microscopic appearance of the cells gave assurance that pure cultures had been obtained.

This method has been very well adapted to the yeasts by Hansen. He has replaced the plates of glass by moist chambers and Böttcher chambers which permit the development of the colonies to be followed by the microscope.

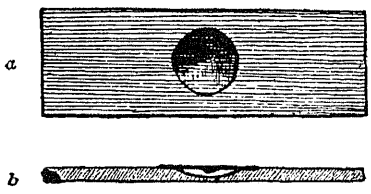


Fig. 65. — Ordinary Moist Chamber.

The ordinary moist chamber (Fig. 65) consists of an ordinary slide, in the middle of which is a depression, covered by a cover slip. On this is suspended a drop of the nutrient solution containing a few cells. The cover slip is sealed with vaseline.

Böttcher's chamber, or the chamber of Van Tieghm and Lecommier, is made up of a glass ring sealed to the slide with Canada balsam. (Fig. 66.) A little water is placed in this little chamber formed by the ring, to maintain the proper humidity. On the top of this ring is placed a cover slip from which is suspended a drop of solution which contains the yeast cells. Vaseline is used to fasten the cover slip to the glass ring. It is necessary to sterilize the apparatus before using by passing through the flame. Precautions must also be observed to prevent the ingress of extraneous microörganisms. This apparatus is very convenient since it allows continued observations of the cell for many days (8 or more).

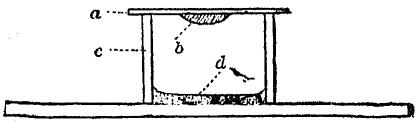


Fig. 66. — Böttcher's Moist Chamber.
a, Cover glass; *b*, droplets of nutrient material; *c*, glass ring; *d*, water.

Hansen's procedure for isolating the cells is to place a drop of gelatin on a cover slip which is ruled into 16 numbered squares. This is then placed over a Böttcher moist chamber. As soon as the gelatin has solidified by cooling, the number of cells in it is counted with the aid of the microscope. This operation is facilitated by the rulings which allow the enumeration of each cell. The number of squares occupied by the cells is determined, after which the apparatus is incubated at 25°. By means of microscopic observations at regular intervals, it is easy to follow the multiplication of the cells as they increase to form colonies. Pure cultures may be secured by inoculating a flask of media with one of these colonies.

Lindner's Method for Securing Pure Cultures: Lindner has devised many methods founded on the same principle but much simpler. One of these known as the drop culture method consists in diluting the yeast until each drop contains about a single cell. Beer wort may be used as the diluent. By means of a pipette with a fine bore, drops of this mixture are placed in the bottom of sterile Petri dishes. Each cell will develop into a colony. From these colonies pure cultures are obtained by means of platinum wires. Transfers are made into nutrient media in order to get the cells in greater quantity. This procedure is not as sure as that of Hansen but is short and serves in many investigations.



Fig. 67. — Mode of Operation for Droplet Cultures according to the Method of Lindner.

Another method devised by Lindner is known as the droplet culture procedure. This is much like the above except that the solid medium is placed on a cover slip which allows continued observations under the microscope.

Determination of the Number of Cells in a Culture and Study of the Multiplication Power of Yeasts: Very often it is desirable to calculate the multiplication power of yeasts or the time required for the cells to divide. One method of doing this is by means of the hemocytometer. This apparatus, devised for counting the corpuscles in the blood, consists of a glass slide upon which is fastened a cylindrical

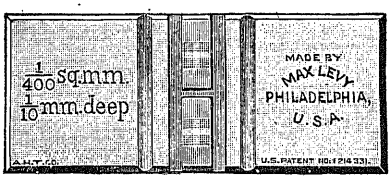


Fig. 68. — Hemocytometer.

or square glass cover slip with a cylindrical hole in the center. Inside this hole is fastened another glass disk which bears a ruled area. When an accurately ground cover glass is placed over the larger cover glass, this disk bearing the ruled portion should be exactly 1 mm. below the bottom surface of it. The ruled area consists of squares of different sizes, depending on the ruling which is used. The value of these squares is usually marked on the end of the slide. While there are many different rulings, the Thoma ruling is as satisfactory as any. (Fig. 68.)

After the yeast solution is carefully shaken to distribute the cells evenly, a drop of it is placed on the disk of the hemocytometer and

the cover glass applied. After the cells have settled, they are counted and the number calculated to the cubic centimeter or millimeter basis. By repeating this operation for several times, some idea may be secured with regard to the progress of yeast development.

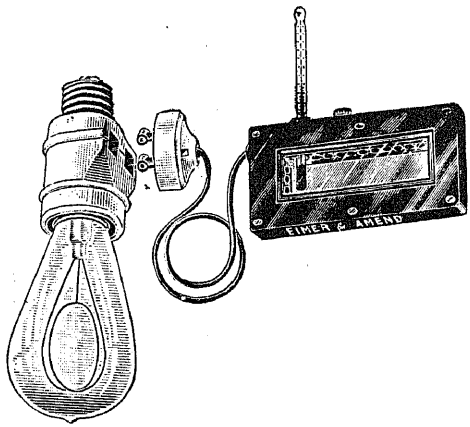


Fig. 68–A. — Warm Stage for Observing the Development of Yeasts.

Slator [1] used the warm stage to determine whether there was a lag phase in the development of yeast. (Fig. 68–A.) By means of this method he was able to follow under the microscope the development of the yeast. He pictures graphically the budding of the yeasts (Fig. 68–B) and found that there was a short lag-phase in the yeast development which lasted for two hours, after which the cells reproduced at the usual rate. For spores, the lag phase was longer.

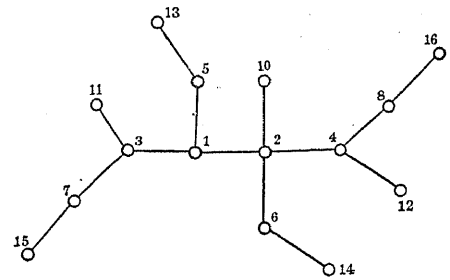


Fig. 68–B. — Slator's Method for Observing Yeast Development on the Warm Stage.

Methods of Studying the Yeasts

Observation of Development in Moist Chambers: The observation of yeasts presents no serious difficulties and the details will not be outlined here. The simplest method is the one used by early workers, Ehrenberg, Mitscherlich, Kützing, Schulze, and Brefeld; it consists in diluting the culture of yeast to such a point that each drop contains only a few cells. A drop of this culture is put on a cover slip which is placed over a moist chamber and incubated at 25° C. The various changes which take place in this drop may be followed on the microscope. Observations may be extended, if desired, for a long time.

The use of the ordinary moist chamber, and especially that of Böttcher, is more convenient. It makes it possible to observe a single cell or a small number of cells for a period of 8 days without danger

[1] Slator, A. Some observations on yeast growth. Biochem. Jour. 12, No. 3, 248–258, 1918.

of contamination. The use of the ruled cover glass on which each square is numbered, makes it possible to follow closely each stage in the development of the yeast. One is thus able to follow the modifications which occur at regular intervals. By this method the phenomena of budding, sexuality, sporulation, germination of spores, etc., may be watched. When this apparatus is used for the study of germination of spores, certain difficulties are encountered. In a yeast which sporulates there are always a few cells which have not formed ascospores. When transferred to a new medium, the asporogenous cells develop more rapidly. Thus, for example, a dilution of beer yeast placed in a Böttcher moist chamber may include asporogenous cells mixed with ascospores. Their early development hinders observation. The simplest method to get around this is to kill the vegetative cells with heat;[1] the spores being more resistant will pass through such treatment.

In order to carry out this, one should proceed as follows: A portion of yeast growth from solid media (agar, gelatin, carrot, etc.) is spread, by means of a spatula, on a sterile cover slip. This is then placed in an incubator at 55–60° for 12 hours. The vegetative cells are not able to withstand this temperature and only the ascospores survive. The yeast is then moistened with a drop of water and a drop is placed in a Böttcher moist chamber. The vegetative cells will remain in the solution but should cause no difficulty since that will not germinate. Guilliermond found this method a convenient one for studying the germination of ascospores.

Lindner[2] extolled very much a method which he devised and termed the adhesive culture. This method simply consists in applying a thin layer of the yeasts or other organisms to a cover slip which is eventually placed in a humid chamber. Let us suppose that we wish to study the bacteria in our saliva. All that would be done would be to apply the tongue to a sterile cover slip. The organisms remain adherent to the cover slip and develop in their own natural medium. The colonies may be sufficiently well isolated for picking pure cultures by means of a platinum wire. In this way, this procedure may be

[1] Another method by which the same results may be obtained has been devised by Hansen. This investigator has stated that the vegetative cells, perhaps on account of their age, are killed by a period of one minute in absolute or 50 per cent alcohol. The ascospores, in a state of maturity, resist the alcohol for a long time. This constitutes a simple method of getting rid of the vegetative cells when only ascospores are desired. (Hansen, Ueber die tötende Wirkung des Aethylalkohols auf Bakterien und Hefen. Cent. Bakt. 45, 1907.)

[2] Lindner, P. Die Adhäsionkultur, eine einfache Methode zur biologischen Analyse von Vegetationsgemischen in natürlichen oder künstlichen Nahrsubstraten. Zeitschr. Spir. Industrie, No. 46 and 47, 1901.

used for isolating species or varieties. Another example would be the investigation of the organisms in the coating of decayed grapes in the fall. A drop of sterile water would have to be placed on the cover slip and a grape pressed into it. This method is very convenient, requires no special media and facilitates microscopic examinations of the organisms. Such a method lends itself to photomicrography. In fact, Lindner [1] secured such illustrations with which to illustrate his book on fermentation microörganisms. The colonies develop very slowly, which lessens the danger of impure cultures.

Methods for Investigating the Cytology of Yeasts: At this time we shall refer to the technique of histology, especially those methods which are of much service. The first step in the cytological examination [2] of yeasts should be a microscopical study of the cells colored with neutral red. To do this, the living cells should be placed in an aqueous solution (1–10,000). The protoplasm and the nucleus will remain uncolored; only the less vital parts of the cell will fix the dye, that is, the vacuole and the metachromatic corpuscles contained in it. The dye will diffuse into the vacuole and stain it very lightly.

When careful observations have been made on living cells, with and without coloration, one may undertake deeper investigations in cells which have been fixed with many of the available substances. The most convenient procedure for this is to cut out a portion of the gelatin or carrot upon which the yeast is developing and place it in the fixing bath. When fixation is completed, the yeast may then be placed on a cover slip, upon which has been spread a layer of gelatin to make it stick. The mount may then be plunged into a staining bath. The fixing and staining will vary, depending upon whether one wishes to study the nucleus or the other contents of the cell.

For investigations on the nucleus, Guilliermond recommends fixation in Bouin's picroformol solution or Perenyi's solution, which have the following composition.

Picroformol solution:

Saturated picric acid	75 parts
Glacial acetic acid	5 "
Formol	20 "

[1] Lindner, P. Atlas der mikroskopischen Grundlagen der Gärungskunde. 2nd Edition, P. Parey, Berlin, 1910.

[2] Guilliermond, A. Recherches cytologiques sur les levures et quelques moisissures à form levures, thèse doctorate ès sciences (Resume in Revue générale de Botan. 15, 1902). Recherches sur la germination des spores et sur la conjugaison dans les levures. Rev. générale de Botanique, 17, 1905. Remarques sur les récents travaux parus sur la cytologie des levures et quelques nouvelles observations sur le groupe de champignons. Cent. Bakt. 26, 1910.

Perenyi's Fluid:

Chromic acid, 5 per cent	3 parts
Nitric acid, 10 per cent	4 "
Alcohol, 95 per cent	3 "

The period of fixation ought to be about 12 hours. Finally, staining is accomplished with Heidenhain's ferric hemotoxylin (mordanting with a $2\frac{1}{2}$ per cent solution of ammoniacal ferric alum, washing rapidly in water and staining in a 1 per cent aqueous hematoxylin solution). By this procedure a satisfactory differentiation of the nucleus is obtained. The basophile grains stain quickly but the metachromatic corpuscles generally do not. The hematoxylin method of Delafield gives good result after fixation in Bouin's solution. These methods at once allow the differentiation of the nucleus, which appears with a diffuse tint, and the metachromatic granules, which take on a wine color. The preparations thus prepared ought to be preserved in Kayser's gelatin-glycerol mixture in preference to Canada balsam which always causes a contraction of the cells.

Alcohol, formalin and Lenhossek's fluid [1] are the most useful fixing solutions for the metachromatic granules; they are not to be recommended so highly for the nucleus. Unna's polychrome blue, Cresyl's blue and methylene blue, employed in 1 per cent aqueous solutions, allow differential staining of the metachromatic corpuscles but generally differentiate the nucleus quite badly. The preparations obtained by these dyes decolorize rather quickly in the gelatin-glycerol solution and are able to be preserved only in Canada balsam.

To demonstrate glycogen, Lugol's solution (iodin in potassium iodide) may be used. For fats, Flemming's solution [2] should be used. The preparations are fixed in it and the fat globules are browned by it.

Methods for Determining the Properties of Yeasts towards Sugars: It is often very desirable, especially when investigating a new yeast, to determine its action towards sugars. The action of yeasts towards such hydrocarbons constitutes a very important step in their differentiation. The simplest method is that devised by Lindner.[3] It

[1] Lenhossek's Fixing Fluid:

Mercuric chloride (sat. in water)	75 volumes
Absolute alcohol	20 "
Acetic acid	3 "

[2] Flemming's Solution:

Osmic acid, 1 per cent in water	15 parts
Crystallized acetic acid	1 "
Chromic acid, 2 per cent in water	4 "

[3] Lindner, P. Mikroskopische Betriebskontrolle in den Gärungswerben. Paul Parey, 6th Edition, Berlin. 1909.

consists of filling an ordinary moist chamber with a drop of yeast water containing the microörganisms. By means of a sterile platinum wire a little of the carbohydrate which one wishes to study, is added to the drop. It may be necessary to pulverize the sugar in order that, as far as possible, equal amounts of the sugar may be transferred. The moist chamber, thus prepared, is covered with a cover slip and placed in an incubator at 25°. The next day the preparation is examined. If a fermentation has taken place, the cover slip may be forced up from the glass collar upon which it rested and a bubble of gas may be seen. (Fig. 69.) In order to make certain that the bubble is made up of carbon dioxide, in part it is sufficient to allow a few drops of caustic potash to fall on the cover slip. If the bubble is CO_2 it will contract and disappear. If, on the other hand, there is no fermentation, the cover slip will not have changed place. It will be adherent to the glass slide.



Fig. 69. — Fermentation in an Ordinary Moist Chamber (after Lindner).

Bronfenbrenner and Schlesinger's Method for Determining the Action of Microörganisms on Carbohydrates: These investigators [1] have proposed a method for studying the action of bacteria towards carbohydrates which may be of value for the yeasts. The method may be outlined as follows: One prepares medium containing 1.5% of agar, 0.5% NaCl, and 1% peptone. This mixture is brought to boiling and the reaction not adjusted. At this point a suitable amount of indicator is added and the medium distributed into small tubes containing 1 or 2 cc. of medium, autoclaved and stored on ice. When used, the medium is melted and to it is added 0.1 or 0.2 cc. of a 20% lactose solution. While hot, this medium is deposited in drops on the inner surface of the bottom of a sterile Petri dish. This may be placed symmetrically by marking the outside of the dish. Each of these drops is inoculated from the suspected colonies or material, leaving two drops uninoculated on each plate as controls. After this, a fresh drop of the medium is placed over the inoculated drops, giving conditions of slightly lowered O tension favorable to carbohydrate metabolism of bacteria. In order to prevent the volatilized acids formed in some drops from causing a color change in other drops, filter paper saturated with NaOH was placed in the top of the Petri dishes. When desired, sterile slides with a concave well may be used. The hollow of the slide is filled with lactose agar, prepared as outlined above, and a sterile cover glass placed over it. This method greatly decreases the length of time required for the formation of gas.

[1] Bronfenbrenner, J., and Schlesinger, M. J. A Rapid Method for the Identification of Bacteria Fermenting Carbohydrates. Amer. J. Pub. Health, 8 (1918), 922–923.

Klöcker's Method for Estimating Alcohol in Fermented Solutions: Klöcker [1] has modified the Pasteur drop reaction for the determination of alcohol in fermented solutions and claims to be able to determine the presence of alcohol in 0.002 per cent by volume. Five cubic centimeters of the solution are used in a vessel 180 mm. long and 24 mm. in diameter. The solution is slowly warmed over a wire gauze by means of a gas flame taking care to prevent bumping. Characteristic oil drops accumulate in the glass tubing, higher up or lower, depending on the concentration of alcohol. By this method it was demonstrated that small amounts of alcohol were formed in yeast water on standing. Other substances, such as acetone, may give the reaction but it is necessary that they be present in large amounts. As a control iodoform may be formed by the following method: A small amount of sodium carbonate (2 grams per 10 c.c. of product) and iodine (0.1 gm.) may be added and the temperature brought to 60° C. until the iodine has disappeared. On cooling, crystals of iodoform will be formed which may be examined under the microscope.

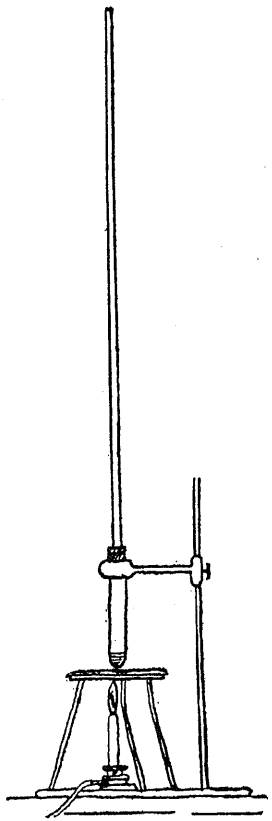


Fig. 69–A. — Klöcker Apparatus for Determining the Presence of Alcohol in Fermenting Solutions.

Determination of Efficiency of Yeast: It is often convenient to have information concerning the efficiency of yeasts especially if it is necessary to select, from among a number of these organisms, one which will cause the maximum amount of change in the shortest time. Different methods may be used. Any of the products of fermentation may be measured quantitatively. The alcohol may be determined at any time during the process of the fermentation but has this disadvantage that considerable time would be used if many determinations were necessary. Probably the most convenient method which has been devised is to measure the amount of carbon dioxide which is formed and from this to determine the extent of the fermentation.

This may be carried out either volumetrically or gravimetrically.

[1] Klöcker, A. The determination of traces of alcohol in fermenting solutions. Cent. Bakt. Abt. II, 31, 108–111; Chem. Absts. 6 (1912), 136.

If one wishes to use volumetric methods, an apparatus must be arranged which will collect all of the gas as it is formed during the fermentation. Such an apparatus was arranged by Slator.[1] An ordinary nitrometer will be sufficient and should be filled with mercury to prevent absorption of the gases, which would occur if water or other liquids were used.

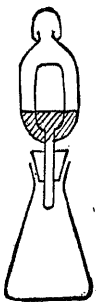


Fig. 69-B. — Meissl Ventilation Valve for Determining the Fermenting Ability of Yeasts (Euler-Lindner).

Euler and Lindner[2] have described the Meissl ventilation valve (see Fig. 69–B) which may be used to allow the carbon dioxide which is formed during fermentation to escape but which retains the water. A small amount of concentrated sulfuric acid is put into the valve to act as an absorbent to retain moisture. The formation of carbon dioxide may be followed by the loss in weight at various intervals. These losses in weight may be plotted according to the time at which they occurred in such a way that the curves may be made which express the fermenting ability of each yeast. By means of these, one is able to compare the yeasts under examination quickly and to determine which is the most "efficient." Alwood[3] has devised a similar valve which is used in the same manner as the Meissl valve. (See Fig. 69–C.) Other devices may be resorted to for reaching the same end.

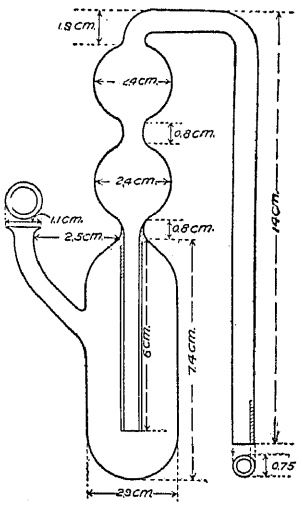


Fig. 69-C. — Detail of Alwood Fermentation Valve.

Preservation of Yeasts: It is often advantageous to keep yeasts over a long period of time without having to transfer them to fresh media very often. Such is the case with laboratory collections. Then, again, it is often desirable to exchange cultures between laboratories and in many cases the distance is great and months are required to cover it. Explorers have had need of preserving the yeasts which have been collected in the countries which they visited. According to the investigations of Hansen,[4]

[1] Slator, A. Jour. Chem. Soc. 89 (1906), 128.

[2] Euler, H., and Lindner, P. Chemie der Hefe unter der alkoholischen Gärung. Leipzig, 1915.

[3] Alwood, W. B. The fermenting power of pure yeast and some associated fungi. U. S. Dept. Agriculture, Bureau of Chemistry, 111–1908.

[4] Hansen, E. C. Recherches sur la physiologie des ferments alcooliques. Comp. Rend. du lab. de Carlsberg, 13, 1898.

the best method for preserving yeasts consists in cultivating them in a 10 per cent sucrose solution with acid. The sucrose does not ferment and is used very slowly. Of 42 yeasts subjected to this method only two have been encountered which did not withstand such a solution. *S. Ludwigii* did not keep longer than 2 years, often 6, and *S. Monacensis* has not survived more than two or three years. The others have been kept for from 13 to 17 years. Generally speaking, the method is a satisfactory one. The Hansen flask is generally used. (Fig. 70.) Jörgensen has suggested a modification of this flask which prevents evaporation.



Fig. 70. — Hansen's Flask for Culturing Yeasts.

Will [1] has proposed another method which consists of drying the yeast and mixing it with powdered silica, plaster of Paris and carbon, the whole being dried at 40° and sealed hermetically. By this method certain yeasts have been kept for 9 years; Hansen has shown that the yeasts form ascospores during this period. We have seen that desiccation is unfavorable to yeasts and the cells form ascospores.

In other investigations, Will [2] has shown that the preservation of the yeasts depends upon three factors, 1, the quantity of yeast, 2, the composition of the medium, and 3, the temperature.

[1] Will, H. Einige Beobachtungen über die Lebendauer getrockneter Hefe. Zeit. f. d. Ges. Brau., 19 and 27, 1896–1904. Beobachtungen an Hefekonservation. Cent. Bakt. 24, 1909.

[2] Will, H. Beobachtungen über Vorkommen leben- und vermehrungsfähigen zellen in sehr alten Würze Kulturen von untergärigen Bierhefen. Cent. Bakt. 44, 1916.

CHAPTER VII

METHODS FOR THE CHARACTERIZATION AND IDENTIFICATION OF YEASTS

NOW that the methods for isolating the yeasts have been outlined, it is proper to investigate the procedure for determining whether a yeast which one has isolated is a new variety or whether it has been described before, and if it has, in what genus it belongs. We have not an easy task before us. When discussing the morphology of the yeasts, it was pointed out that it was very difficult to distinguish between them. Their shapes are very much the same, varying between a sphere, ellipse and cylinder. With rare exceptions the form and structure of their ascs, and the appearance of their ascospores do not present specific characters. On the other hand the morphological characteristics are not constant but subject to variation. The shape and dimensions of the cell vary with the age, the physical and chemical conditions of the environment. It is, then, rather difficult to find in the morphology of the yeast the differential characteristics which permit a close separation of varieties. It becomes necessary to search for distinctive characters among the varieties. We shall have to look to the macroscopic appearance on solid media, to the appearance of the scum on liquid media in contact with air, to the variations produced by the action of various media, and especially, to the biochemical characteristics of the variety. Hansen's investigations have shown that the shape of the cell, the dimensions, and the appearance are, in themselves, sufficiently reliable factors for the identifications of species. To him we owe the solution of this question on specification. To his work, we must look for a great number of characteristics and a method for differentiating between species with all the security desirable. Hansen has used as determining characters the shape and dimension of the cell at different temperatures and in different media, the shape of the ascospores and their method of germination, the limits of temperature for budding, the formation of a scum, sporulation, macroscopic appearances of the scum and of cultures, the biochemical properties and especially their action toward different carbohydrates. Lindner has added to these the very convenient characters determined from the "giant colony."

We shall now take up in detail the various characteristics which

are necessary for the identification of yeasts. To simplify matters, let us suppose that we have isolated a yeast which we wish to identify, to determine whether it is a new variety or whether it is a variety already known.

Character of the Vegetation in the Sediment

The preliminary examination ought to be concerned with the sediment. Finally the microscopic features of the cells should be investigated.

Characteristics of the Sediment: The microscopic investigation of the sediment of yeast growth ought to give very useful data. It may be able to remain distributed through the medium or fall to the bottom. Possibly it will attach itself to the sides of the culture flask.

Shape and Dimensions of the Cell: The second step in the examination should be the microscopic examination of the cells taken from the sediment of a culture in carbohydrate media. Hansen recommended for this study a young culture grown at 25° for 24 hours or for 3 or 4 days at room temperature. The dimensions of the cells are variable characteristics. Beauverie[1] has recently applied biometric methods to the yeast. For this 100 cells are taken from a culture and the measurements plotted against the corresponding cell. In this way a curve may be drawn and a polygon made which will express the frequency of certain sizes. From the appearance of this polygon, one may characterize the species.

The genus *Saccharomycodes* is easily distinguished by the elongated tubular cells and their mode of multiplication, which is intermediate between budding and fission. In the same way, it is not difficult to distinguish a budding yeast from the *Schizosaccharomyces*. Certain yeasts of the genera *Torulaspora* and *Debaromyces* and many varieties of the *Torula* possess a sufficiently characteristic spherical shape with a great globule of fat. Other yeasts are elongated or cylindrical and bud at their extremities. (Fig. 4.)

Aside from these yeasts which we have mentioned and which possess some morphological characteristic to differentiate them, the great majority of the yeasts are not so characterized. Most of them may not be separated by some quick microscopic feature. Some of them may be grouped together by their shape but no separation may be made between them by it. The microscopic examination of a yeast tells us nothing about its genus or family.

Optimum Temperatures and Limits for Budding: With regard to budding there are maximum, optimum and minimum temperatures

[1] Beauverie, J. Les méthodes de la biométrique appliquée à l'étude de levures. Comp. Rend. Soc. Biol. 1912.

which do not coincide with those for sporulation, scum formation, and which serve to distinguish between the yeasts. These temperature studies are, then, very useful in identifying yeasts. Below are listed the results of temperature determinations for a few varieties of yeasts cultivated in beer wort.

Name of Yeast	*Maximum Temperature* °C.	*Minimum Temperature* °C.
Saccharomyces cerevisiae	40	1 to 3
Saccharomyces pastorianus	34	0.5
Saccharomyces intermedius	40	0.5
Saccharomyces validus	39–40	0.5
Saccharomyces turbidans	40	0.5
Saccharomyces Marxianus	46–47	0.5
Willia anomala	37–38	1.5
Saccharomyces Ludwigii	37–38	3–1
Johannisberg yeast II	37–38	0.5

An inspection of this table is very instructive. One sees that certain varieties are able to live at high temperatures (*S. Marxianus* 46–47°); others, on the contrary, are not able to bud below 34° C. It is also seen that a determination of the temperature limits enables the separation between varieties of the same shape (*S. pastorianus* and *Saccharomyces intermedius*). It has been shown that when yeasts are cultivated at temperatures approaching their maximum temperatures, there is a tendency to take the shortest, or spherical, form (Hansen and Klöcker).

Thermal Death Point Determinations

By this is meant the amount of heat which is necessary to destroy the yeast. This varies, of course, depending on whether it is tried on vegetative cells or spores. It has been stated that the ascospores are very resistant, more so than the vegetative cells. It is then necessary to determine, for each variety, the thermal death point for the spore and the vegetative cell. The temperatures which are thus observed vary with the age of the culture and the condition of the cell. A great many factors influence determinations of the thermal death point of microörganisms. Some of these are the reaction of the medium, the time of exposure, the presence of organic matter, the presence of spores, etc.

Temperature Limits and Optimum for Ascospore Formation

It has been shown in a preceding chapter that temperature plays an important rôle in this phenomenon. The investigations of Hansen

have shown that ascospore formation has very definite limits of temperature, and outside of these temperatures, none are formed. Certain temperatures are especially favorable (optimum). For a given temperature, situated between these limits, the ascospores form most abundantly. Hansen has also shown that at the temperature limits and the optimum the duration of ascospores formation varies with the variety. He regards these as very important characteristics. It is, then, feasible to determine for each variety the following:

1. The temperature limits, maximum and minimum:
2. The optimum temperature.
3. The temperatures at which ascospores form between the temperature limits.

To secure these characteristics with accuracy, the various yeasts are placed in a carefully controlled incubator. They should be under the same conditions and on plaster of Paris blocks. The number of hours or days which are necessary for the first rudiments of ascospore formation to appear, should be noticed.[1]

Hansen has expressed graphically by means of a curve the results secured with six varieties: *S. Pastorianus, cerevisiae, ellipsoideus, validus, intermedius* and *turbidans*. The temperature of ascospore formation was expressed on the abscissa and the time of ascospore formation on the ordinate. The curves for all of the varieties were identical. The curves were convex towards the temperature axis. They are limited since they do not go beyond the temperature of ascospore formation. When studied relatively, it can be noted that their convex parts are little different; on the other hand their extremes are quite distinct from one another. These are, then, the temperatures which it is important to note well.

The time necessary for ascospore formation in six varieties under the same conditions of temperature is equally interesting to consider. At the maximum temperature ascospore formation is accomplished in about 30 hours; at the optimum temperature, there is little difference among the different varieties; at the lower temperatures, the differences are more and more striking. Thus, for example, *S. cerevisiae* does not develop ascospores at 11.5° until about 10 days, *S. intermedius* at the end of 77 hours, etc.

The following tables will indicate the limits of temperature, the optimum temperature and the time necessary for ascospore formation of six varieties studied by Hansen.

[1] How one is to tell when ascospore formation has begun is variable. It will be necessary to adopt some criterion for the beginning of the ascospores.

MAXIMUM, MINIMUM AND OPTIMUM TEMPERATURES FOR THE FORMATION OF ASCOPORES OF SIX VARIETIES STUDIED BY HANSEN

Name of Yeast	*Maximum Temperature*	*Optimum Temperature*	*Minimum Temperature*
Saccharomyces cerevisiae	37–35	30	9–11
" *Pastorianus*	29–31.5	27.5	0.5–4
" *intermedius*	27–29	25	0.5–4
" *validus*	27–29	25	4.8–5
" *ellipsoideus*	30.5–32.5	25	4.7–5
" *turbidans*	33–35	29	4–8

TIMES AT WHICH ASCOSPORES BEGIN TO FORM IN SIX VARIETIES STUDIED BY HANSEN

Name of Yeast	*Maximum Temperature*	*Optimum Temperature*	*Minimum Temperature*
Saccharomyces cerevisiae	29 hours	20	10
" *Pastorianus*	30	24	14
" *intermedius*	34	27	17
" *validus*	35	28	9
" *ellipsoideus*	36	21	11
" *turbidans*	31	22	9

Sexuality: Morphological Characteristics of the Asc and Ascospores. Germination of the Ascospores

The copulation which precedes the formation of the asc in certain yeasts, the morphological characteristics of the asc and ascospores, and, finally, the mode of germination have great significance in the determination of varieties. Thus, the existence of a copulation served Barker in creating the genus *Zygosaccharomyces*, characterized only by their sexuality. With the exception of the *Schizosaccharomyces* which possesses an analogous copulation but in which the form and mode of cellular division do not allow any confusion, all of the yeasts in which the asc results from a copulation fall into the *Zygosaccharomyces*. It is true that Klöcker has discovered a new variety from which he has made a new genus, *Debaromyces globosus* and in which he has observed sexual phenomena of the same order. This variety, however, is distinguished from all of the other yeasts by the special shape of its ascospores, upon which Klöcker has founded the genus *Debaromyces*. Therefore if one encounters a yeast which indicates sexual processes in the formation of the asc, and if this yeast divides by budding and does not form ascospores with a special form, it may be placed with some certainty with the *Zygosaccharomyces*.

In *Nematospora coryli* and *Monospora cuspidata* the ascs are larger and possess a more elongated form than the vegetative cells.

Finally a fixed number of ascospores is possible, 4 in the former and 1 in the latter. Among the other yeasts, (*Schwanniomyces, Torula,* etc.) it has been pointed out that the asc forms after an apparent copulation.

Sometimes the shape of the ascospore is characteristic: hat shaped in *Willia anomala,* with rings in *Willia Saturnus,* with a knotty membrane in *Schwanniomyces.* Thus, the existence of sexual phenomena in the formation of the asc, the shape of the asc and ascospores are sufficient to characterize the *Nematospora, Monospora, Zygosaccharomyces, Debaromyces, Schwanniomyces* and *Willia.*

The method of germination of the ascospores often furnishes delicate information for the determination of species. Hansen's researches have made it possible to characterize the *Saccharomyces,* in which the ascospores undergo generally a copulation at the beginning of germination, for they are connected two by two with a copulation canal. Two new cells are formed by a process intermediate between budding and fission.

But for the great number of varieties, notably most of the industrial yeasts, the shape and dimensions of the ascospores and their germination do not offer any distinctive factor which may be utilized for their identification.

Temperature Conditions which Influence Scum Formation and Microscopic and Macroscopic Characteristics

The mode of formation and the appearance of the scums formed by different yeasts make excellent characters upon which to separate the yeasts. By this method, as has been discussed in Chapter IV, two groups of yeasts may be distinguished. The yeasts of one group form a scum at the beginning of fermentation; it is their mode of vegetation; the scums are well developed, grayish, with folds, and usually dry. They contain air. This group includes the genera *Willia* and *Pichia.* For this group the temperature limits of budding and scum formation are evidently the same.

However, many of the yeasts do not form a scum at temperatures close to their temperature limits. It has been stated that Klöcker has demonstrated the beneficial effect of alcohol in the medium on scum formation in the genera *Pichia* and *Willia.*

The yeasts in the other group form their scums very slowly after the principal fermentation has terminated. The scums are rather viscous, wet, and do not contain entrained air. Many of the yeasts in this group form only a ring and some form neither ring nor scum. The character of the scum of this group has served Hansen for sep-

arating species. Hansen has shown that scum formation is related to the temperature. Certain temperature limits exist, minimum and maximum. They vary with the species and are easy to determine. When once determined, they are very useful for the separation of species.

The scums have different macroscopic characteristics, depending on the variety and the temperature of culture; it may cover the surface entirely, float about as a small island, or appear as a ring around the walls of the container. In most cases the cells which make up the scum are united end to end to give somewhat the appearance of a rudimentary mycelium. In some of the bottom yeasts and the industrial varieties, one may observe the presence of durable cells. On the scum, then, one should determine the temperature limits, the optimum temperature, the macroscopic appearance of the scum at different temperatures and the microscopic appearance of the cells which make up this scum at different temperatures. The relation of temperature to scum formation is a very important characteristic. This determination furnishes important data. The following tables, prepared from data secured by Hansen, are interesting.

TEMPERATURES AT WHICH SCUM FORMATION TAKES PLACE WITH SIX VARIETIES AS DETERMINED BY HANSEN

Name of Yeast	*Maximum degrees*	*Optimum degrees*	*Minimum degrees*
Saccharomyces cerevisiae	33–34	20–22	6–7
" *Pastorianus*	26–28	26–28	3–5.8
" *intermedius*	26–28	26–28	3–5
" *validus*	26–28	26–28	3–5
" *ellipsoideus*	33–34	33–34	6–7
" *turbidans*	36–38	33–34	3–5

TIMES AT WHICH SCUMS BEGIN TO APPEAR IN THE SIX VARIETIES STUDIED BY HANSEN

(Time expressed in days)

Name of Yeast	*Maximum degrees*	*Optimum degrees*	*Minimum degrees*
Saccharomyces cerevisiae	9 to 18	7 to 10	2 to 3[1]
" *Pastorianus*	7 to 10	7 to 10	5 to 6[1]
" *intermedius*	7 to 10	7 to 10	5 to 6[1]
" *validus*	7 to 10	7 to 10	5 to 6[1]
" *ellipsoideus*	8 to 12	8 to 12	2 to 3[1]
" *turbidans*	8 to 12	3 to 4	5 to 6[1]

It is evident that *Saccharomyces ellipsoideus* is distinguished from *Saccharomyces turbidans* by its maximum temperature. On the

[1] Months.

other hand, *Saccharomyces intermedius, validus* and *Pastorianus,* varieties equally closely related, have the same temperature limits. With regard to the time necessary for the scum to appear, some equally interesting differences are brought out.

Macroscopic Appearances of Cultures on Solid Media [1]

The various varieties of yeasts do not develop after the same manner on solid media (agar and gelatin). They offer vegetative growths which we may use as differential characteristics.[2] Certain varieties liquefy gelatin rapidly, others slowly or not at all. This is an important characteristic. It is important to inoculate the yeast into agar, gelatin, carrot or potato, and examine the microscopic appearance of the growth after the yeast has developed. The following determinations may also be made.



Fig. 71.—Plate Culture.

Plate Culture: This is prepared by putting a little of the yeast in dilution into a Petri dish. The dish is partially filled with gelatin which serves as a food. When the gelatin has solidified, each cell will develop into a colony which, for each yeast, will have some differential characteristic.

Streak Culture: A test tube or Petri dish containing a solid medium with a large surface is streaked with a little of the yeast. The yeast will develop by growing along this line of inoculation.

Stab Cultures: The yeast is stabbed into a solid medium by means of a stiff platinum wire. This introduces the yeast into an environment which has a reduced air supply.



Fig. 71-A.—Stab Culture in Gelatin.

One may thus obtain many characteristics which will serve in the differentiation of the yeasts. The colonies will possess special forms. Hansen, for instance, has shown that on beer wort gelatin, *S. cerevisiae, ellipsoideus, Pastorianus, validus,* and *intermedius* when inoculated in streak cultures, present very different appearances to the naked eye. The same was found out with regard to the stab cultures.

[1] The Descriptive Chart of the Society of American Bacteriologists has been used by some investigators in America for recording the salient characters of yeasts. It has the advantages of offering a uniform method of procedure and of recording concisely in a small space the data for each yeast. The comparison of characteristics of yeasts is thus made easy.

[2] According to the investigations of Orsos, the form of the colonies is a function of the elasticity of the medium upon which the yeasts are. The state of cohesion of the substrate is one of the determining factors and also, to a lesser degree, the activity of the yeast (Orsos, Die Form. der tierfliegenden Bakterien und Hefencolonien. Cent. Bakt. 54, 1910).

Giant Colonies

Lindner [1] has devised another method which yields very good differential characteristics. This involves the growth of giant colonies which are much utilized today by bacteriologists and mycologists. They are made by inoculating a large surface of gelatin at a single point. The giant colonies grow steadily until they have reached larger proportions than the ordinary colony. The inoculation is accomplished by placing a drop of dilution in the middle of a large surface. At laboratory temperatures (20° C.) it will require two months for the colony to reach its large proportions. There is an optimum temperature for each variety which permits the most characteristic form. Giant colonies, in each case, give a very different appearance. (Fig. 72.) However in most cases they merely furnish characteristics of the group and not specific characteristics. Giant colonies are sometimes susceptible to variations.

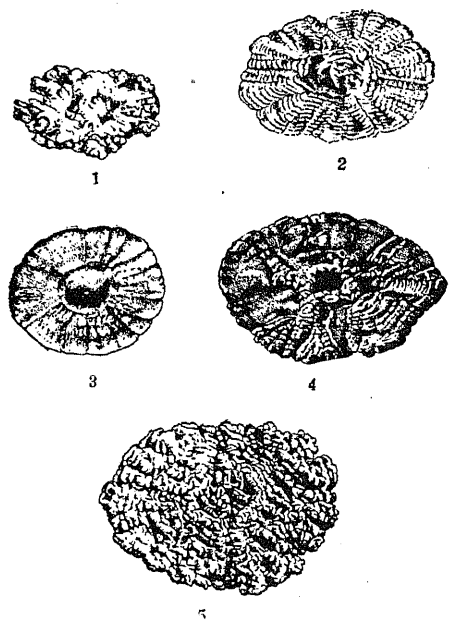


Fig. 72. — Appearance of Giant Colonies of Various Yeasts.

1, *S. Pastorianus;* 2, *S. intermedius;* 3, *S. ellipsoideus;* 4, *S. turbidans;* 5, *S. validus* (after Lindner).

The types of the cells in the mediums also furnish valuable information. *Saccharomyces Ludwigii, S. marxianus, carlsbergensis* and *P. membranaefaciens* form mycelial filaments. *Saccharomyces Bailii* produce ameboid cells (Fig. 31).

Biochemical Activity of Yeasts

The action of the yeasts towards the different sugars is valuable information in their differentiation. The method of Lindner,[2] outlined above, may be used. It should be determined whether the yeast de-

[1] Lindner, P. Das Wachstum der Hefen auf festen Nährboden, Wochenschr. Brau. 10, 1893.

[2] Lindner, P. Gärversuche auf vershiedenen Hefen und Zuckerarten. Wochensch. Brau. No. 17, 1900.

composes sugars like saccharose or maltose, and whether it ferments others as saccharose, maltose, galactose, fructose, dextrose, lactose, raffinose, melibiose, methylglucoside, dextrine, inulin, etc. This differential action towards the various carbohydrates is important in the determination of the yeasts. Some will decompose dextrose, others will not. The great majority will not decompose lactose. Lactose-fermenting yeasts are not uncommon, however. Beijerinck[1] (1889) found such a yeast in the Kefir grain. One was also found in Edam cheese by the same author. He called the one from the cheese *Saccharomyces tyrocola* and the one from the Kefir grains *Saccharomyces kefir*. Grotenfeld[2] in the same year found a lactose-fermenting yeast in milk. With regard to these yeasts being true saccharomyces there seems to have been some difference of opinion, since others have been unable to detect the formation of ascospores. Bochicchio[3] isolated a non-spore bearing yeast from Grana cheese which he named *Lactomyces inflans-caseigrana*. Freudenreich and Jensen[4] report a lactose-fermenting yeast from Emmenthaler cheese. Jensen[5] later found two such yeasts in butter. Maze[6] when studying ten *Torulae* from cheese found only one which fermented lactose. The others fermented many of the common carbohydrates. Duclaux reported three lactose fermenters. Hunter[7] isolated such a yeast from "foamy" cream and regarded it as the essential organism for this abnormality. The thermal death point of the yeast seemed to be near 55° C. Typical spores were not demonstrated which would seem to exclude it from classification with the *Saccharomycetes*. Trehalose is rarely fermented by the yeasts. Hansen has been able to subdivide the *Saccharomyces* into six groups according to their action on the carbohydrates. He has used such characteristics as the production of acetone and other compounds.

Industrial yeasts are often classified as bottom or top yeasts. In the beer industry, the top fermentation and the bottom fermentation are brought about by certain variations in the method of manu-

[1] Beijerinck, M. W. Die Lactase ein neues Enzyme. Cent. Bakt. Abt. I., 6, 44, 1889.

[2] Grotenfeld, G. Studien über die Zersetzung der Milch. Cent. Bakt. Abt. I, 5, 607, 1889.

[3] Bochicchio, N. Ueber einen Milchzucker Vergärenden und Käseblahungen hervorrufenden neuen Hefepilz. Cent. Bakt. Abt. I, 15, 546.

[4] Freudenreich, E. V., and Jensen, O. Ueber die Einfluss des Naturlabes auf die Reifung des Emmenthalerkäses. Cent. Bakt. Abt. II, 3, 544.

[5] Jensen, O. Studien über Ranzigwerden der Butter. Cent. Bakt. Abt. II, 8, 251.

[6] Maze, P. Quelques nouvelles races de levures de lactose. Ann. Past. Inst. 17, II.

[7] Hunter, O. W. A lactose-fermenting yeast producing foamy cream. Jour. Bact. 3, 293–300, 1918.

facture. One class is able to live at higher temperatures while the other demands a lower temperature. This characteristic does not seem to be constant, for a top yeast may transform itself into a bottom yeast.

Finally, certain other characteristics, as the amount of alcohol produced in a fermentation, parasite or saprophyte, or pathogen, may be used. Lindner has also shown that one may use the nitrogenous and hydrocarbon metabolism properties of the yeast.

Methods for the Characterization of the Torula, Mycoderma and Pathogenic Properties

By summing up all of the characters, one may arrive at a determination of a true yeast. But when a yeast is encountered which does not form spores or a scum, as many of the industrial yeasts and pathogens, or if it grows on the surface but forms no ascospores as with the *Mycoderma*, the determination becomes more complex, if not impossible. The most important characteristics are the temperature of scum and ascospore formation. The biochemical characteristics and the giant colony formation remain.

It is almost impossible to recognize most of the pathogenic yeasts described in the last few years. Many of these varieties need more study according to the newer methods. Leberle and Will [1] have shown that many of the characteristics for the differentiation of the *Mycoderma* and *Torula* should be taken from the biochemical properties of the species: assimilation of various sugars, alcohol, organic acids, resistance of the various varieties towards alcohol and oxidations of these compounds. Lutz and Guegen,[2] from their work, have proposed another method for the determination of the species which consists in microscopic and macroscopic examination of the yeast on a great many different media. They propose to use the following media:

I. General Media.
 A. Raulin's solution, acid and neutral.
 B. Gelatin prepared from Raulin's solution.

II. Nitrogenous media with organic nitrogen.
 Raulin's solution with urea in place of the ammonium nitrate.

III. Media made up of different carbohydrate materials and polyatomic alcohols.

[1] Will, H. Beitrage zur Kenntniss der Sprosspilze ohne Sporenbildung. Cent. Bakt. 19, 1907; 221, 1908; Beitrage zur Kenntniss der Gattung Mycoderma nach Untersuchungen von Hans Leberle. Zeit. Brauw. 28, 1910.

[2] Lutz, L. and Guegen, F. De l'unification des methodes de cultures des Mucidinees et des levures. Bull. de la Soc. de Mycologie de France, 1901.

A. Raulin's solution without carbohydrates to which various sugars are added.

IV. Media containing hydrocarbons.

A. Starch or inulin added to Raulin's solution.

V. Various media.

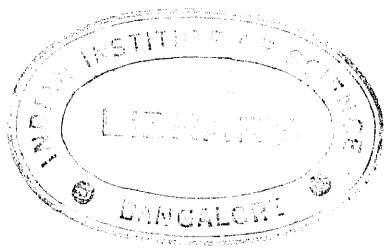
A. Milk

B. Potato

C. Carrot

D. Egg albumin

This method has not been used sufficiently to clearly judge its value.


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CHAPTER VIII

VARIATION OF SPECIES

THIS is a rather intricate question to consider. The characters which we have just studied may be utilized for the determination of a species when they are fixed and do not vary within the species. This involves the whole question of constancy of characters. Are such characteristics absolutely constant or only relatively constant? To what extent may they vary? Do well-determined varieties of yeasts exist or may they change with the environment? Finally, if these variations occur, are they permanent or simply transitory? There is the important question, for if the characteristics upon which we would determine a yeast vary, all hope of differentiating species becomes illusory.

Again it is Hansen [1] who has contributed the most to elucidate this problem by showing that yeasts may undergo more or less important variations; some permanent, others transitory. In some cases changes have taken place which have been of such nature that the yeast has not returned to its former state, even after attempts covering a number of years. Let us consider some of these variations which permit the determination of species and separate them from each other. Some will be found to be more constant; others variable. We shall distinguish variations in shape (morphological) from variations in function. In this category, we shall separate the variations which are temporary from those which are permanent. Certain it is that such a division is arbitrary because modifications in morphology are always accompanied by modifications of physiological activities. It is well to adopt it, however, for the convenience of exposition.

Morphological Variations: Polymorphism: Yeasts are quite polymorphic and may show different shapes in the same culture. This may depend upon the conditions which surround the yeasts.

If one inoculated, for example, a single cell into a nutrient medium, it would be found that many different cells would develop from this single cell; from this, it is seen that the yeasts have no definitely constant shape. With *Saccharomyces cerevisiae* it has been

[1] Hansen, E. C. Experimental studies on the variations of yeast cells. Read before the Botanical section of the British Association. Ipswich, Sept. 13, 1895. Annals of Botany, 9, 1905; Ueber die Variation bei den Bierhefepilzen und bei anderen Saccharomyceten. Zeit. Brauw. 21, 1898. Cent. Bakt. Abt. II, 4, 1898; Recherche sur la phys. et la morphologie des ferments alcool. C. R. des trav. du lab. de Carlsberg, 5, 1900.

shown that the cells may pass from the round shape to oval and even elongated and curled cells. A great difference will also be seen in the dimensions of the individual cells. In old cultures, yeasts are generally smaller, on account of the scarcity of food which does not permit the young cells to become fully developed. Also, such cultures will give ascospores which germinate into smaller cells. We have also pointed out how the cells of *S. apiculatus* may lose their apiculate shape during a few generations. Hansen has shown that the temperature may play a rôle in influencing the shape. For example, in cultivating *Saccharomyces carlsbergensis* in beer wort at 27° and 7° C. this author obtained two very different shapes. Those which formed at 27° C. presented a normal appearance; the others, formed at 7° C., were very curious colonies made up of elongated cells forming a sort of mycelium. The ascospores, themselves, may present among the various individuals of the same species very different shapes. With *P. membranaefaciens*, for example, the ascospores are quite spherical at times, and at others, may be egg-shaped.

All of this simply points out that the cells in yeasts are not constant in shape and may, depending upon the circumstances, take on variable forms temporary or permanent. In a word, they are polymorphic. Although a species may present these various forms, there usually is a predominant shape which, to a certain degree, is characteristic and may be regarded as normal for the species under question. In certain cases, one may note the predominance of abnormal forms among the normal.

Hansen has taken two series of cultures in which the cells are distinctly different from a single cell of *S. Carlsbergensis*. One series shows oval or round cells of the *cerevisiae* type while the other is of elongated cells, more like the *Pastorianus* type. These last vegetations are, then, abnormal, although they persist through a series of cultures. Hansen has been able to preserve this variation for six months. It appears, then, that in the life of the yeast diverse variations may spring up which may endure for a time and give the yeast an abnormal appearance. These are the spontaneous variations which occur without apparent cause and which may persist for a certain period of time and recall the fluctuations or fluctuating variations which are so often encountered with the higher plants and animals.

Permanent Variations: Aside from the temporary variations there are permanent ones which persist through a number of generations and very often become absolutely constant, creating new varieties. The investigations of Lepeschkin [1] offer examples of more

[1] Lepeschkin, W. Zur Kenntniss der Ehrlichkeit bei der einzelligen Organismen. Cent. Bakt. 10, Abt. II, 1903.

constant variation in the characteristics of yeasts. Guilliermond has observed in a young culture of *Schizosaccharomyces Pombe* in beer wort a certain number of abnormal mycelial forms which suggest the appearance of little specks scattered in the growth among ordinary cells. (Fig. 73.) These have been isolated and obtained in pure culture and maintained constantly in the same mycelial structure. Lepeschkin has also isolated a similar mycelial structure which appeared in the growth of a young culture of *Sch. mellacei* developing in glucose yeast water. (Fig. 74.) These mycelial forms in *Sch. mellacei* are either with or without spores. They make up, then, a constant species incapable of transforming themselves into ordinary cells and seem to result from an hereditary modification of the cells. This transformation, caused without apparent cause, seems to fall into the category of de Vries' *mutations*. In the sporulation of yeasts we often find variations. Yeasts seem to lose very easily the power of forming ascospores and often do not recover it. Definite asporogenic races of yeasts are thus formed. Hansen made the first observations on this subject.

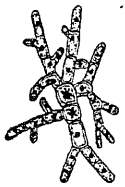


Fig. 73. — Mycelial Formation in *Sch. Pombe* (after Lepeschkin).

In isolating a large number of cells of *S. Ludwigii*, this author obtained three different races; one is marked by its ability to form ascospores; another group is made up of yeasts in which this power is almost extinct; the last contains yeasts in which it has entirely disappeared. There are, then, three races, an asporogenic, a feebly sporogenic and a sporogenic. The asporogenic race can be maintained for a long time.

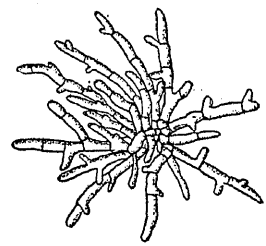


Fig. 74. — Mycelial Formation in *Sch. Mellacei* (according to Lepeschkin).

On the other hand, Lindner [1] has shown that when *S. Bailii*, *P. hyalospora* and *P. farinosa* are cultivated for a long time on must gelatin they lose completely their ability to form spores. Holm has reported the same thing with cultures of *Saccharomyces multisporus*, cultured for a long time on beer wort with sucrose. Beijerinck [2] has secured similar results to those of Hansen with *Sch. octosporus*. In cultivating this yeast on nutrient gelatin, this investigator noticed three types of colonies; first, white colonies made up of cells which do not produce ascospores; secondly, light brown colonies made up

[1] Lindner, P. Mikroskopische Betriebskontrolle in den Gärungswerben, Paul Parey, edit. Berlin, 6th edition 1909.

[2] Beijerinck, M. W. Weitere Beobachtungen über die Octosporushefe. Cent. Bakt. 3, 1897.

of a mixture of sporogenic and asporogenic cells; thirdly, clear brown colonies made up of only asporogenic cells. The asporogenic cells could be maintained constantly in this state. Beijerinck effected a separation of the types by heating at 56°. The asporogenic type was killed by this treatment, only the spores passing through. These when grown on gelatin produced sporogenic cells with only about 1 per cent of asporogenic cells. These latter cells increase in proportion as the cultures are kept in the laboratory. The yeast slowly changes into an asporogenic type.

Both of these types present different physiological and morphological characteristics. The sporogenic type is made up of cells more elongated, and liquefies gelatin more quickly than the asporogenic variety, which presents round cells grouped like the *Sarcina*. Both varieties may be recognized macroscopically when treated with iodine. The colonies made up of sporogenic cells are blued since the membranes of the ascospores are impregnated with starch while the asporogenic colonies are colored yellow. This is, then, a very definite example of a transformation of a yeast to a permanent asporogenic type.

Similar results have been secured by Beijerinck (?) with *Sch. Pombe*. By cultivating this yeast on nutrient gelatin, this investigator noticed the formation of two kinds of colonies, one white and composed of sporogenic cells, the other brown, and made up of asporogenic cells. Here, then, we have both the sporogenic and asporogenic cells. The loss of sporulation may be accompanied by a loss of sexuality as has very often been noticed in the yeasts and about which a little has been said in a preceding chapter. This is true in a yeast secured from Beijerinck's laboratory under the name of *Sch. mellacei*, in which the ascs form from ordinary cells without undergoing any copulation. This yeast, which differs a little from *Sch. mellacei*, seems to be a parthogenetic variety. Quite a similar observation has been reported with *S. Ludwigii*. Guilliermond had the opportunity to observe two types of this yeast from the same source. Both came from Hansen's laboratory. With one the ascospores underwent a copulation at the moment of germination as is the normal procedure for this species. In the other, the copulation had entirely disappeared. All of the variations of sporogenic function and sexuality which we have mentioned up to this point supervene without apparent cause and may be regarded as true mutations.

Other investigations by Hansen on the loss of sporulation give us an example of variation produced by an accurately determined cause. It is known that the maximum temperature of budding in a variety of yeasts, is always a few degrees higher than the maximum tempera-

ture of sporulation. Inversely the minimum temperature of budding is always a little lower than that for sporulation. What happens, then, if one allows the yeasts to remain for a period between these two temperatures? Such is the question that Hansen tried to solve. He obtained a complete loss of the ability to sporulate by cultivating a number of the yeasts for generations in beer wort at a temperature higher than the maximum for sporulation. He could not obtain the same results by placing the yeast at a temperature lower than the minimum for sporulation. The transformation is accomplished slowly and by successive culturing; the number of sporogenic cells gradually diminish until they totally disappear. Thus may be obtained asporogenic varieties which may be maintained indefinitely. Hansen has been able to keep them for sixteen years without taking up the sporogenic property again. The types of yeasts thus secured may be regarded as constant. These varieties offer new characteristics which give evidence of profound modifications in the structure of their protoplasm. The power of budding often increases and the colonies present a different appearance than yeasts. On the other hand, all of the varieties thus obtained, with certain rare exceptions, seldom produce a scum. Thus the loss of power to form spores is a characteristic definitely acquired by this variety when cultivated for a certain time on beer wort at a maximum temperature for the formation of endospores.

This transformation of a species which is sporogenic into an asporogenic type is a most typical example of an acquired characteristic which may approach the attenuation of a virus, as shown by Pasteur. How Pasteur attenuated the *Bacillus anthracis* by growing it at a temperature of 42–43° C. is well known. Not only the toxic properties of the bacillus disappeared but also its sporogenic functions. Here we have the creation of a new type characterized by the loss of virulence and ability to form spores.[1]

How does this loss of ability to sporulate in yeasts operate? Is it a transformation or a selection? Hansen does not regard it as a selection because many of the cultures of yeasts which he used to produce this sporogenic type, especially the yeast *Johannisberg II*, contains only sporogenic cells. Numerous observations have convinced him that an asporogenic cell exists in this culture. It must be a typical transformation.

Hansen has shown that by varying the composition of the medium

[1] Hansen has approached this transformation of a sporogenic into an asporogenic yeast by certain variations which have been observed in the higher plants. In America the banana reproduces asexually while in mid-Asia it reproduces sexually.

and, for example, employing a solution made up of peptone, maltose, and various salts or a must gelatin, that the composition of the medium does not play any rôle in this transformation. The same is true for aeration of the culture. The only factor which seems to have any effect is the temperature.

The formation of sporogenic and asporogenic types of yeast in the same culture of yeast seems rather common. Nadson, rather recently, has observed asporogenic varieties in *Nadsonia fulvescens*. The colonies of this type have a white color which distinguishes them from the sporogenic colonies which are reddish.

Saito has noticed in *Zygosaccharomyces Mandshuricus* the formation of asporogenic types, indicated by a transparent yellow color, while the sporogenic types have a white color. The asporogenic type appears as a mutation. If the white colonies are isolated both sporogenic and asporogenic colonies are obtained. When the asporogenic colonies are isolated, one obtains, almost exclusively, asporogenic yellow colonies. There seems to be a tendency to return to the sporogenic type as is shown by other data. The sporogenic type is distinguished from the asporogenic type by a certain number of characteristics. The asporogenic type contains but a small amount of glycogen. Their reaction towards Lugol's iodine allows them to be distinguished macroscopically. On the other hand the asporogenic variety liquefies gelatin while the sporogenic race does not. The sporogenic type forms a deposit of spherical cells at from 28° to 30° while the other type forms long cells sometimes in chains.

This is contrary to the observations of Hansen on *S. pastorianus* and the yeast Johannisberg II, in which the sporogenic race was only slightly formed; in *S. mandshuricus*, as in *Sch. octosporus* and *Nadsonia*, the asporogenic varieties appeared quickly and do not seem to depend on conditions of culturing but on internal conditions. A low temperature, however, as in *Schizosaccharomyces octosporus*, favors the formation of asporogenic races, and in old cultures the asporogenic types seem to predominate.

Saito has also observed the formation of asporogenic races in *Zygosaccharomyces Mandshuricus*. There are white colonies and grayish yellow colonies with irregular surfaces. The inoculation of a white colony produces a majority of white colonies with a few yellow colonies. The inoculation of grayish yellow colonies gives the asporogenic cells. The asporogenic race is distinguished from the sporogenic race by the shape of its cells, longer and arranged in chains with less glycogen. The white race which is not definitely asporogenic has a tendency to lose its sexuality and to give parthenogenetic ascs after unfruitful attempts at copulation.

Physiological Variations: Besides morphological variations, one may also observe physiological variations. A yeast may, for example, under certain conditions, induce more or less active fermentations in the same way that a bacterium may be made more or less virulent. But while certain bacteria, *Bacillus anthracis* for instance, may be made avirulent, among the yeasts it is impossible to suppress the fermenting function. One may decrease it or even increase it but not entirely blot it out.

The first investigations on variation of physiological nature in yeasts were carried out by Hansen. When cultivating two races of *Saccharomyces carlbergensis* for a long time in two series, one on ordinary beer wort and the other on the same substance to which gelatin had been added, he was able to build up a more actively fermenting type on the gelatin medium. By cultivating the ascospores of *Saccharomyces cerevisiae* in gelatin with yeast water, the same investigator obtained a variety which would form from one to three per cent more alcohol than the original culture. On the other hand, by cultivating *Saccharomyces carlbergensis* in must at 32° C. Hansen has obtained a variety which formed less alcohol than the normal. According to Hansen, these results are due to a selection born of a transformation. The most active type will tend to be built up. From all of these examples which we have mentioned one is justified in concluding that cells of the same species of yeast often present great differences and that new varieties may be created by selection which have special physiological properties.

This is the point of departure from the use in the industries of "selected yeasts." By making a series of cultures from a single cell, as each cell possesses slightly different physiological properties, one may obtain strains presenting the definite properties of the original cell. Some will be more feeble and others more active. The latter have been termed "yeasts by selection" for they may be maintained for a longer or shorter period and are then able to yield the best results in the industries.

All of the physiological variations which we have just mentioned, increase or decrease of the fermenting function, are abrupt transformations. It is now time to look into the work of Effront and other workers for examples of transformations due to determined causes, as the becoming accustomed to chemicals.

The work of Beinarcki has shown that the antiseptics in small doses progressively increase the fermenting power of yeast up to a certain limit where the yeast degenerates and dies. There are, then, doses which "favor" this ability up to certain limits. Effront[1] has

[1] Effront, J. L'influence de l'acide fluorhydrique et des fluorures sur les levures de bière. Comp. Rend. Acad. Sciences, 117, 1893; 118 and 119, 1894.

studied the effect of fluorides and hydrofluoric acid on yeasts. A special concentration exists where the vegetative growth is greatest, and also where the fermenting activity is greatest. These two optima do not coincide. When the fermenting power is greatest, the vegetation is absent. Dienert,[1] in about the same manner, has shown that when yeasts which ferment galactose actively are placed in a saccharose solution and eventually, after washing, are placed in galactose, they fail to induce a fermentation quickly. Only after from 24 to 36 hours is a typical fermentation started. If the same experiment is repeated with the exception that galactose replaces the saccharose, the fermentation will start in about an hour. The time for fermentation to start is thus greatly shortened. By the latter treatment, the yeast has been "accustomed" to the galactose by the preliminary treatment.

These results compare with investigations of Duborg.[2] It is known that the greater number of the yeasts are able to ferment galactose. Duborg has been able to train yeasts, which normally do not ferment this sugar, to ferment it. He cultivated his yeasts in a liquid very rich in carbohydrate materials (yeast water, 25 per cent, glucose 5 per cent and galactose 5 per cent). It is the cultivation of the yeast in this solution in the presence of galactose which gives it a power which it did not possess. The more recent investigations of Harden and Norris[3] have confirmed these data.

An increase in the activity of zymase may also be explained by these data. Duborg has gone still further. He claims that a yeast which does not invert cane sugar may be made to do so by cultivating it in a nitrogenous medium containing dextrose and saccharose. This statement has been refuted by Klöcker and Hansen who claim that a yeast which does not ordinarily decompose sucrose cannot be made to do it. According to these authors[4,5] the possession of invertase is a constant characteristic and useful in the determination of the yeasts.

Other investigations on physiological variations have been carried

[1] Dienert, G. Sur la fermentation de la galactose et l'accoutumance des levures a ce sucre. Ann. Inst. Past. 14, 1900.

[2] Duborg, E. De la fermentation des Saccharides. Comp. Rend. Acad. des Sciences, 128, 1899.

[3] Harden, A. and Norris, R. The fermentation of galactose by yeast and yeast juice. Proceedings of the Royal Society, 82, 1910.

[4] Klöcker, A. La formation des enzymes dans les ferments alcooliques peut-elle servir a caracteriser l'espece? Comp. Rend. lab. de Carlsberg, 50, 1909.

[5] Hansen, E. C. Recherches sur la physiologie et la morphologie des ferments alcooliques. XI. La spore de saccharomyces devenue sporange. Recherches comparatives sur les conditions de vegetative croissance et le développement des organes de reproduction des levures et des moisissures. Comp. Rend. du lab. de Carlsberg, 5, Book 2, 1902.

out by Will[1] and Jorgensen.[2] Under industrial conditions, degenerations of yeasts which have been pure when used have often been obtained. A yeast which may always have given good results may, of a sudden, give a beer with evident defects. The fermentation is too slow or too rapid, or the beer takes on an abnormal taste. A degeneration of the yeast has taken place. Will has noticed that this may occur in the cells of scum yeasts more than in those of bottom yeasts. According to Will, the yeast may be regenerated by repeated culturing. On the other hand Jörgensen has arrived at similar conclusions. It seems then, that the cells in scum yeasts are more liable to degeneration.

We shall now consider the results secured by Hansen[3] on the transformation of bottom yeasts into top yeasts. It has been pointed out that, from the industrial viewpoint, yeasts are divided into two groups, bottom and top. The first are those which only produce fermentations at the higher temperatures, the second class produce fermentations at the lower temperatures. Hansen had noticed that certain yeasts of the bottom type, after having been cultivated for a period of time at low temperatures are able to induce top fermentations. He then searched for an explanation of this observation, taking *Saccharomyces turbidans* which is well known as a bottom yeast.

He inoculated a trace of this yeast into flasks containing beer wort and left them for from 3 to 5 months at a temperature of 5° C. after which he transferred some cells from these flasks to others at more favorable temperatures. The yeast thus obtained produced a top fermentation. Of 130 cells which he examined, none produced a bottom fermentation. Hansen has thus obtained the transformation of a yeast which is normally a bottom yeast into a top yeast by simply keeping it at a temperature of 5° C. What is the cause of this transformation? In order to seek an explanation, Hansen analyzed the properties of the cells of a culture of *Saccharomyces turbidans* which were subject to this transformation. He examined 100 cells and found that one-half offered characteristics of a top yeast and one-half had the characteristics of a bottom yeast. He extended his observations by inoculating cells from mixed industrial yeasts into separate flasks and incubating at 5° C. At the end of from 3 to 4 months, the cells of the bottom yeasts had given no growth while, on the other hand, the cells from top yeasts had given evidence of development. The

[1] Will, H. Zeitschr. f. d. ges. Brau. 21, 1898.

[2] Jörgensen, A. Untersuchungen über das Ausarten der Brauereihefe. Zeit. f. d. ges. Brau. 21, 1898.

[3] Hansen, E. C. Oberhefe und Unterhefe. Studien über Variation. Cent. Bakt. 15, 1905; 18, 1907.

cells of the bottom yeast continued to give a bottom fermentation and those of top yeasts a top fermentation. This seemed to show that there was no transformation from a bottom yeast into a top yeast but probably a selection; at a temperature of 5° C., the cells from the top yeast developed alone while the cells of the bottom yeast remained stationary. Their properties however were not modified. *Saccharomyces turbidans* offers, then, as Hansen described in 1883, characteristics of a bottom yeast. Such changes have been induced since that time that part of the cells in a culture cause a bottom fermentation and part a top fermentation. This change had been induced spontaneously while the yeast was in Hansen's laboratory and without apparent cause. Furthermore this characteristic seems to have been retained, since Hansen, on later observations, found the same proportion of cells of both types of yeast. This phenomenon is related to the mutations of de Vries.

Studies on other bottom yeasts by Hansen have also given some evidence of a change from bottom to top yeast. He isolated 1000 cells from the yeast *Johannisberg II*, which, through a number of generations, had given a bottom fermentation. He cultivated these separately; 984 gave a true bottom fermentation while 16 produced an intermediary fermentation. The cells inducing bottom fermentation tended to remain constant while those in the top fermentations tended to be changed.

Two of the 16 cultures which induced an intermediary fermentation, were studied further. Here are the results for one of them: of 100 cells, 5 gave a top fermentation, 55 an intermediary fermentation and 40 a bottom fermentation. Other observations were made on the 5 cells which produced the top fermentation. Of 100 cells, 78 gave a top fermentation, 9 an intermediary fermentation and 13 a bottom fermentation. In this particular case there was a more or less definite movement toward the top fermentation. Similar results have been obtained by using ascospores in place of the vegetative cells.

Saccharomyces carlbergensis and *monascencis* gave similar results.

On the contrary, Hansen did not observe this tendency on the part of the top yeasts to transform into bottom yeasts. They seemed to be much more stable. He was not able to transform *Saccharomyces validus*, a typical top yeast, into one which would produce a bottom fermentation. In one analysis, he could find only 3 " bottom cells " in 100 and he could not increase this number. In another analysis, out of 1529 cells only one was separated which induced bottom fermentation. With *Saccharomyces cerevisiae*, another top yeast, out of 2423 cells, Hansen found only 7 which gave a bottom fermentation and a more careful study of the vegetation formed by these indicated that

they were mixed types which only tended very slightly to induce bottom fermentation.

These experiments show that the distinction set up between top and bottom yeasts does not exist; in reality, one may find both cells of top and bottom yeasts in the same culture. It may be that the conditions in the environment favor the development of one type, as with *Saccharomyces turbidans* incubated at 5° C. The bottom yeast may thus change into a top yeast, and perhaps top yeasts into bottom yeasts.

In summarizing, it is apparent that the same species of yeast may undergo important variations in morphology and function. Thus, a new series of varieties and types may be created in which particular characteristics are maintained for a certain time, be it indefinitely. Thus also, with regard to physiological functions, the fermenting function is susceptible in a certain measure, of being enfeebled or increased, or a top yeast may change into a bottom yeast. Is one justified, in light of these data, in refusing to differentiate between species? Certainly not. We may use the temperature limits for the formation of ascospores, scum and for budding, and the action toward different sugars. The characteristics of the ascospores and germination maintain themselves without undergoing modification. We may conclude that if the species is not definitely constant in yeast, it is as constant as with the other plants. More difficulties are encountered with the yeasts on account of the variability of a great number of characteristics.

"All of the variations of species seem to be more doubted than with the higher plants; variations are more rapid with the yeasts. Further, the specific characters are less definite than in the higher plants and one may distinguish less easily those which are constant and specific from those which are not. One may encounter yeasts scarcely more variable than the higher plants, but their generation time is much shorter and, consequently, the phenomena of variation appear more quickly. Here the investigator may be witness to remarkable transformations in a short time." Such are the words which Hansen has used with regard to this question.

CHAPTER IX

CLASSIFICATION OF THE YEASTS

WE have seen, in the preceding chapters, that the yeasts may be regarded as making up a group of lower *Ascomycetes* closely related to the family of *Endomyces*. It has been shown, also, that they seem to be derived from an ancestral form related to *Eremascus fertilis* which may give birth at times to various representatives of Endomycetes and yeasts. On account of the close relations which exist between the yeasts and Endomycetes, Van Tieghem,[1] in his recent classification, has attempted to place them in one group, the *Eremascines*. Hansen, on the contrary, considers the yeasts as making up a special family of *Ascomycetes* related to *Exoascus* and *Endomycetes* which he calls *Saccharomycetes*. Although among the *Endomycetes* and *Saccharomycetes* there exist all degrees of transition, and although there are such varieties as *End. javanensis*, which is with difficulty attached to either groups of these two families, we believe with Schröter [2] that the yeasts should be made a distinct family closely related to the *Endomycetes* and making up the group *Protoascines*. The great number of the yeasts and their medical and industrial significance seem to justify this opinion. How shall the representatives of the *Saccharomycetes* be grouped? Profiting by the morphological investigations of these later years by Hansen,[3] a classification may be proposed. This classification which was proposed in 1904 and has been added to by the work of Klöcker [4] and Lindner,[5] is today uniformly accepted.

However the recent work on the systematic and phylogenic relations of the yeasts, not considering the great lines of this classification, do not justify it completely. We shall adopt a classification a little different from that of Hansen's.

[1] Van Tieghem. Elements de botanique. Masson et Cie., Paris, 4th Edition, 1906.

[2] Schröter, J. in Engler and Prantl's Die nat. Pflanzen. W. Englemann, Leipzig, 1889.

[3] Hansen, E. C. Grundlinien und Systematik der Saccharomyceten. Cent. Bakt. 12 (1904), 528.

[4] Klöcker, A. Deux nouveaux genres de la famille des Saccharomycetes. Comp. Rend. des trav. du lab. de Carlsberg, 8, Book 4, 1909.

[5] Lindner, P. Mikroskopische Betriebskontrolle in den Gärungswerben. Paul Parey, Edit. Berlin, 6th edition, 1909.

We shall eliminate from the *Saccharomycetes* all of the yeasts which do not form ascospores. Such are the *Torula*, *Mycoderma*, and the pathogenic yeasts. These yeasts do not offer any characteristic which permits giving them an accurate place in classifications of fungi. The one may represent forms derived from mycelial fungi and fixed in the state of yeasts, the others may be true yeasts which have become asporogenic. They may be placed apart in a separate group from the *Saccharomyces*.

In the family of *Saccharomyces*, we shall include all yeasts which sporulate whatever their mode of division. Contrary to Hansen, we shall not separate the *Schizosaccharomyces*. These yeasts, if they are differentiable from the other yeasts by the mode of division (transverse partition), belong incontestably to the *Saccharomycetes* by the copulation which preceded the formation of the asc with the greater part of them. They are related to the *Saccharomycodes* in which the cells divide by an intermediate method between typical partition and budding and which offer a form of transition between the *Schizosaccharomyces* and other yeasts. We shall subdivide the *Saccharomyces* into five groups.

The first group will include the *Schizosaccharomyces* characterized by their method of division, transverse partition. By the formation of the asc which results from an isogamic copulation, this group may be regarded as strictly related to the *Endomycetes*.

In the second group are placed those yeasts which offer in the origin of the ascs, a copulation iso- or heterogamic and which, having lost their sexuality, have, however, preserved traces of it. It is a very primitive group from which seem to be derived all other budding yeasts.

In the third group, we find all yeasts in which the formation of the asc is not preceded by any sexual phenomenon and which in liquid media, vegetate, at first, as a sediment and produce later a scum very slowly more or less mucous. In certain species, a parthenogamy between ascospores may intervene. Almost all of the species in this group are able to induce fermentations. This group corresponds to Hansen's first group less the yeasts of our second group.

In the fourth group are yeasts which, without any trace of sexuality in the formation of the asc, form in liquid carbohydrate media a mycodermic scum. After the air has penetrated into its interstices, it takes on a dry opaque appearance. Most of these yeasts do not cause fermentations but produce ethers. Some of them have parthenogamy between the ascospores. This group corresponds to Hansen's second group.[1]

[1] The classification of Hansen differs from ours only in the following points: First, the *Schizosaccharomyces* are excluded and considered as a special group of

Finally in the fifth group (*Saccharomycetes* doubted by Hansen) we shall include the genera *Monospora, Nematospora,* and *Coccidiascus,* which offer by the shapes of their ascospores very special characteristics and of which the affinities are not well known.

The first group includes only the genus *Schizosaccharomyces* represented by only a few species.

In the second group we shall place the genus *Zygosaccharomyces,* first characterized only by isogamic or heterogamic copulation which precedes the formation of the ascs and which seems to make up with the *Schizosaccharomyces* an archaic type which has retained an ancestral copulation similar to the *Eremascus.* Next comes the genus *Debaromyces* (Klöcker) characterized by its ascopores in thorny membrane. This genus actually includes only a single species *Deb. globosus* which has a copulation similar to the *Zygosaccharomyces* and appears to progress toward heterogamy. The new genus *Nadsonia* (*Guilliermondia*) of Nadson and Kinokotine, created for species with heterogamic copulation, is characterized by the fact that the asc is formed from a cell and a bud from that same cell. The ascospores, generally to the number of one, resemble the ascospores of *Debaromyces.* They have a large fat globule in their center and a membrane slightly verrucose. The genus *Schwanniomyces* (Klöcker) includes only *Sch. occidentalis* which is characterized by a thorny membrane but belted by a projecting collar. Here the ascs have preserved traces of sexual attraction and attempt to anastomose two by two before sporulation. The genus *Torulaspora,* created recently by Lindner for yeasts which present the typical spherical shape of the *Torula,* is badly defined; however, we shall reserve for it a place along with the *Schwanniomyces* because the ascogenic cells show traces of sexual attraction. We shall include in this genus, by the side of *Torulaspora Delbrücki* (Lindner), a certain number of yeasts which offer equally a spherical shape and which, on the other hand, have preserved traces of sexual attraction (yeasts E and F of Rosa for instance).

The third group, one large in numbers, includes the genus *Saccharomycodes* (Hansen) in which the cells multiply by a process intermediary between transverse partition and budding, and which, from this point of view, may be regarded as a form of transition

yeasts. Secondly, the *Saccharomyces* are divided into two groups only; the first includes yeasts which form a scum only at the end of fermentation. This scum is mucous without occluded air bubbles. This group includes the genera *Saccharomyces, Zygosaccharomyces, Saccharomycodes, Saccharomycopsis.* The second group includes the types which give a scum at the beginning with bubbles of air in it: genera, *Willia* and *Pichia.* Thirdly, the genera *Nematospora* and *Monospora* make up a group under the name of doubtful Saccharomycetes.

between the *Schizosaccharomyces* and the ordinary yeasts. This genus is characterized by a tendency to produce mycelial formations rather well developed and the replacement of ancestral sexuality by a compensating phenomenon or parthenogamy consisting in the fusion of ascospores two by two.

After this genus may fall the genus *Saccharomycopsis* (Schiönning) which only includes *S. guttulatus* and which is characterized by ascospores in a double membrane resembling those of the *Endomycetes* (*Eremascus*, *End. fibuliger*, and *capsularis*). We shall separate the *Saccharomycopsis capsularis* (Schiönning) from this genus in order to include it with the genus *Endomyces*. The investigations of Guilliermond with this species seem to indicate that it approaches *E. fibuliger* when the mycelial formation and method of formation of the ascs are considered.

The genus *Saccharomyces* (Meyen) includes all yeasts in which the formation of a mycelium is not observed and in which sexuality has disappeared with the exception of a few species (yeast *Johannisberg II*) in which the primitive copulation has been replaced by a fusion between the ascospores (parthenogamy).

Finally the genus *Hansenia* (Lindner-Klöcker) characterized by its special apiculate cells and hat-shaped ascospores terminates the series.

Since the *Saccharomyces* includes all brewery, distillery, cider and wine yeasts and other industrial yeasts, and consequently a large number, we shall, with Hansen, make six sub-groups according to their fermentation reactions: First, *Saccharomyces* which ferment saccharose, maltose and dextrose with no action on lactose. Secondly, *Saccharomyces* which ferment saccharose and dextrose but do not ferment lactose or maltose. Thirdly, *Saccharomyces* which ferment dextrose and maltose but not saccharose and lactose. Fourthly, *Saccharomyces* which ferment dextrose but neither lactose, saccharose, or maltose. Fifthly, *Saccharomyces* which ferment lactose. Sixthly, *Saccharomyces* which produce no fermentation and in which the fermenting function is imperfectly known.

In the fourth group, we shall include, with Hansen, two genera, *Pichia* (Hansen), characterized by hemispherical ascospores, and *Willia*, characterized by special-shaped ascospores having the form of a derby hat.

In the fifth group, we shall place the genus *Monospora* (Metschnikoff), characterized by its ascs with a single ascospore, and the genus *Nematospora* (Peglion), characterized by its asc with 8 fusiform ascospores with a long mycelium and the genus *Coccidiascus* (Leger), which is characterized by a probable copulation preceding the forma-

tion of the asc containing 8 ascospores. These two genera, until their relations are better known, merit a place apart.

Along with the *Saccharomycetes* we shall make up a family of non-*Saccharomycetes* or doubtful yeasts — all those which do not form spores. Three groups will be made here: First, the *Torula*, including all yeasts which in liquid media vegetate in the bottom of the culture tube but eventually form a slimy scum with no air bubbles, having all of the other characteristics of the third group with the exception of spore formation. Secondly, the genus *Pseudosaccharomyces* proposed by Klöcker for the apiculate yeasts which do not sporulate and the *Mycoderma* which forms a slimy scum with air bubbles. These correspond, in general, with the fourth group of the *Saccharomycetes*. Thirdly, the genus *Medusomyces* (Lindau), characterized by a thick, stratified, gelatinous scum, and the pathenogenic yeasts to which have been given the generic name of *Cryptococcus* (Vuillemin).[1] Below is given a résumé of the classification which we have just outlined.

Family of Saccharomycetes

Unicellular fungi, multiplying by budding, sometimes by partition and forming ascs. Each cell may change into an asc in which are formed from 1 to 4, rarely 12, ascospores, each ascospore enclosed in a vegetative cell.

First Group

Yeasts multiplying by partition. Ascs often derived from a copulation, with 4 or 8 ascospores. These are provided with a single membrane.

Genus I. Schizosaccharomyces

Second Group

Budding yeasts; sexual phenomena, sometimes only in traces, in the formation of the asc.

[1] De Beurmann and Gougerot (Les mycoses dans le nouveau Traité de médecine et de therapeutique de A. Gilbert et Thoinot, Baillière, ed. Paris, 1910) have created the following three genera for pathogenic yeasts which do not sporulate.

1. Atelossaccharomyces (ατελος = imperfect) which include all well-differentiated yeasts which do not sporulate.

2. *Parasaccharomyces* which include fungi resembling the yeasts but which offer rudimentary filamentous forms sometimes true filaments.

3. *Zymonema* (ζύμη = levure, νῆμα = filament) which include intermediate forms between the yeasts and *Endomycetes* characterized by a mixture of yeast forms and mycelial formations.

The pathogenic yeasts are, as stated above, not very well known and the placing of them into genera is difficult. This classification seems premature.

Genus II. Zygosaccharomyces. Barker

Ascs preceded by a copulation, iso- or heterogamic, ascospores with a thick membrane.

Genus III. Debaromyces. Klöcker

Ascs derived from a copulation most often heterogamic, with globular ascospores provided with a single verrucose membrane.

Genus IV. Nadsonia. (Guilliermondia) Nadson

Ascs derived by budding from a cell formed by heterogamic copulation. Ascs with walls more or less thick.

Genus V. Schwanniomyces. Klöcker

Traces of copulation; ascospores with a single verrucose membrane formed of two unequal parts girdled and provided with a projecting collar.

Genus VI. Torulaspora. Lindner [1]

Round cells resembling *Torula* with a large fat globule in the center. The ascs present only traces of copulation in their origin.

Third Group

Budding yeasts which form, in sugar solutions, at first a deposit, and later on a more or less slimy scum without occluded air. Ascospores, round or oval, with from 1 to 2 membranes, germinating by budding; generally produce alcohol.

Genus VII. Saccharomycodes. Hansen

Cells divide by a procedure intermediary between budding and division. Frequently rudiments of a mycelium with transverse walls.

Ascospores in a single membrane germinating in a single direction in the form of a tube which swells up and separates the ascospore by the formation of a transverse wall accompanied by a slight circular constriction. Germination often preceded by parthenogamy.

Genus VIII. Saccharomycopsis. Klöcker

Ascospores in two membranes.

[1] This genus seems to be characterized by its traces of copulation as the investigations of Rose have indicated. We have united these characteristics with those provided by Linder to characterize this genus.

Genus IX. Saccharomyces. Meyen

Ascospores in a single membrane, germinating by budding sometimes with the formation of a rudimentary mycelium.

First Sub-Group: *Saccharomyces* fermenting dextrose, maltose and saccharose, but not lactose.

Second Sub-Group: *Saccharomyces* fermenting dextrose and saccharose but neither maltose nor lactose.

Third Sub-Group: *Saccharomyces* fermenting dextrose and maltose but neither saccharose nor lactose.

Fourth Sub-Group: *Saccharomyces* fermenting dextrose but not maltose, saccharose nor lactose.

Fifth Sub-Group: *Saccharomyces* fermenting lactose.

Sixth Sub-Group: *Saccharomyces* not inducing fermentations or in which the characteristics of fermentations are insufficiently known.

Genus X. Hansenia. Lindner. Klöcker

Apiculate cells. Ascs with a single ascospore.

Fourth Group

Budding yeasts which form a scum immediately in sugar media; scum is dry and opaque, including air. Ascospores in characteristic shapes (in the form of a lemon, hat or angulous) with a single membrane, often with a projecting collar. Generally do not produce alcohol but ether.

Genus XI. Pichia. Hansen

Hemispherical ascospores. Rudimentary mycelium well developed. Do not cause fermentations.

Genus XII. Willia. Hansen

Ascospores in the form of a lemon or hat with a projection like a girdle; generally do not produce alcohol but ether.

Fifth Group

Yeasts in which the relationships are not well known.

Genus XIII. Monospora. Metschnikoff

Budding yeasts, ascs with a single ascospore, in the form of a needle, germinating laterally by budding.

Genus XIV. Nematospora. Peglion

Budding yeasts, ascs with 4 fusiform ascospores, terminating with a cilium.

Family of Non-Saccharomycetes

Budding yeasts but forming no ascs.

Genus I. Torula. Turpin

Generally spherical cells, often forming a scum but only after fermentation; scums always slimy without the presence of air bubbles.

Genus II. Pseudosaccharomyces. Klöcker

Apiculate cells.

Genus III. Mycoderma. Persoon

Cells generally elongated; scums are formed at the end of development with the presence of air bubbles.

Genus IV. Medusomyces. Lindau

Scums appearing at the beginning thick, stratified and gelatinous.

Genus V. Cryptococcus. Kützing-Vuillemin

Yeasts without ascs, parasitic to animals.

There now remains a descriptive study of species of yeasts. We shall describe all of the yeasts which are actually known. The yeasts are excessively numerous and space will not permit an examination of all of them. Many of the industrial yeasts, well known on account of their physiological properties, have not been studied morphologically and have not been given provisional names. Finally many of the *Torula* and *Mycoderma* and pathogenic yeasts have been insufficiently characterized. It will be necessary to pass over those which are not completely described and devote our attention to those which are more fully characterized.

PART II—STUDY OF SPECIES

CHAPTER X

FAMILY OF SACCHAROMYCETACEAE

UNICELLULAR fungi, multiplying by budding or transverse division and forming ascs. Each cell has the ability to change into an asc and form from one up to twelve ascospores, each ascospore germinating and forming a vegetative cell.

First Group

Genus I. Schizosaccharomyces

Round or rectangular cells, dividing by transverse partition. Asc with 4 or 8 ascospores ordinarily resulting from isogamic copulation.

SCHIZOSACCHAROMYCES OCTOSPORUS. Beijerinck[1]

This species was found by Beijerinck on fruits from warm climates (raisins from Corinth, Greece, Asia Minor, and Turkey, and figs from Smyrna). It possesses large cells of various shapes; some are rectangular, resembling the oidia of Endomyces or giant bacteria; others are spherical and resemble the Micrococcus (Figs. 8 and 14). The rectangular cells predominate in young cultures, while the spherical cells appear especially when multiplication commences, and change into an asporogenic type.

Often the cells near the ends show the presence of circular lines which mark the divisions between the old part of the cell wall and that which was newly formed.

Multiplication is brought about by transverse division: a wall appears in the middle of the cell, making two daughter cells. The wall quickly increases in size and the two cells become round in shape. The cells may remain attached in such a way that the mother cell may have 4 or more daughter cells attached. The daughter cells may undergo a transverse partition without separating from the mother cell. The cells are then grouped somewhat in the same way as the *Sarcina*.

Sch. octosporus never contains glycogen at any time of its de-

[1] Beijerinck, W. *Sch. octosporus.* Cent. Bakt. 16, 1896, also 1897.

velopment. Sporulation seems to be rapid and appears at the en two or three days on solid media (slices of carrot, beer wort or to which gelatin has been added). It may happen that, at the of fermentation, the vegetative cells in the sediment may con ascospores but, in this case, sporulation is feeble. The ascs f with difficulty on plaster of Paris blocks; according to Seiter appear at the end of six or seven hours at 25° C.

Sporulation is preceded by a sexual phenomenon which studied by Guilliermond[1] in 1901. The asc results from an isoga copulation which takes place between two neighboring cells. T unite by means of a copulation canal through which the content the two cells mix. The fusion results in formation of a large oval zygospore (6– wide and 14–20.5 long). This transfo slowly into an asc. Sometimes the fu remains incomplete, and the asc seems t formed of two enlarged parts united b canal. All intermediary stages are fou however, between complete and incomp fusion (Figs. 14, 15, and 16).

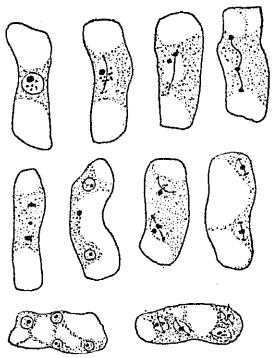


Fig. 74–A. — Karyokenesis in the Ascs of *Schizosaccharomyces Octosporus.*

The ascospores, always 4 or 8 in e asc, are usually ellipsoidal in shape. T are surrounded by a membrane, cove with a starchy reserve material which st blue with iodine (Lindner); this is util during germination. The wall of the asc persists, or more often di pears immediately before germination; the ascospores, having been free, separate or remain attached. Germination begins by a swel of the ascospores which take the appearance of vegetative cells divide in the usual manner. On nutrient gelatin the colonies are ro with a thick center.

We have seen that Beijerinck noticed, when the yeast was i ulated onto gelatin, that three sorts of colonies were obtainable; f white colonies made up of cells which formed ascospores; secon clear brown colonies made up of only vegetative cells; thirdly, l brown colonies made up of cells forming ascs and those with no a

[1] Guilliermond, A. Recherches histologique sur la sporulation des Sc saccharomycetes. Comp. Rend. Acad. Sciences, 133, 1901; Recherches cytologi sur les levures et quelques moisissures à form levures. Thèse for the Doct in Sciences at the Sorbonne, Storck, Lyon, 1902. Summarized in the R generale de Botanique, 15, 1903; Recherches sur le développement du *Gloeospo nervisequum* et sa prétendue transformation en levures. Rev. gen. de Bot. 1909.

This indicated the possibility of two types of yeasts, a sporogenic and a non-sporogenic race. The light brown colonies are formed by a mixture of the two types.

Both types are encountered constantly in nature. They possess different morphology and physiology. The sporogenic race is made up of rectangular cells while, on the contrary, in the asporogenic type, round cells predominate and are often situated in the shape of a *Sarcina*. Finally the sporogenic race liquefies gelatin more rapidly than the asporogenic. The sporogenic race shows a tendency to transform into the asporogenic type when cultivated for a long time in the laboratory.

Schizosaccharomyces octosporus never produces a scum on beer wort but simply a feeble ring. It ferments lactose, dextrose, levulose, d-galactose, d-mannose, raffinose, dextrine and α-methylglucoside. It may also cause a feeble fermentation of xylose (Lindner). It has no action on saccharose which it does not ferment. From the biochemical point of view, *Schizosaccharomyces octosporus* is distinguished from *Saccharomyces mellacei* and *Sch. Pombe* in that it ferments d-galactose and has no action on saccharose nor inuline.

SCHIZOSACCHAROMYCES POMBE. Lindner

Schizosaccharomyces Pombe was discovered by Saare and Zeidler in African beer made from millet and described by Lindner [1] in 1893. Its cells are much smaller than those of *Schizosaccharomyces octosporus*, usually retangular, with rounded ends and about 7 μ in length and 4.5 μ broad. The cells resemble the Oidia of the Endomyces very much or even giant bacilli. In old media they tend to decrease in length and approach the appearance of bacteria.



Fig. 75. — Vegetative Cells in *Schizosaccharomyces Pombe* on Carrot Slants.

They divide by transverse partition always in the same way as *Schizosaccharomyces octosporus* (Fig. 75). The transverse walls separate the cells into unequal parts. Under certain conditions, as the absence of air, the cells elongate very much and may present many cross walls without any separation taking place. Sometimes one may observe the formation of lateral branches. We have seen that Lepeschkin has been able to obtain on beer wort, in the deposit, little flocs having a characteristic mycelial formation with cross walls and branchings. The cells of this yeast never include glycogen. Growth demands at least a temperature of 15° C.

[1] Lindner, P. *Schizosaccharomyces Pombe no. sp.* ein neuer Gärungserreger Wochenschr. Brauerei. 1893.

Sporulation is accomplished with difficulty on plaster blocks, but is easily observed in hanging drops in which it appears at the end of seven or nine hours. It is easily obtained on slices of carrot at the end of a few hours, in old cultures on gelatin, and in the growth in beer wort after fermentation. Guilliermond [1] has found that the asci result from an isogamic copulation. The fusion is always incomplete and results in the formation of two swelled parts united by a narrow canal. The ascospores, always to the number of four, are formed in pairs, two in each swelling. Their dimensions are about 4 μ in diameter (Fig. 76). Their walls are covered with a starchy substance which is colored blue with iodine. Quite often the ascospores may form at the expense of cells which have not undergone copulation. The asci reabsorb their membranes generally before germination, and thus free the ascospores. Germination is brought about in the following manner: the ascospores swell up and divide by transverse partition like the vegetative cells.

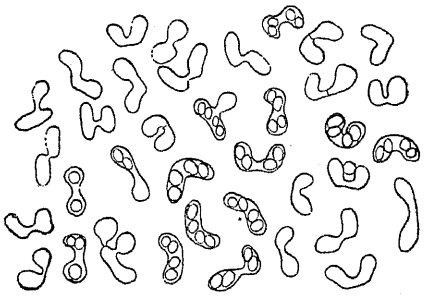


Fig. 76. — Formation of the Asc in *Schizosaccharomyces Pombe.*

Beijerinck has noticed as in *Sch. octosporus,* the existence of a sporogenic variety forming white colonies on gelatin and an asporogenic variety forming brown colonies on the same medium.

This yeast forms no scum on beer wort but produces a ring at the end of a month. On gelatin, there develops a compact layer of fine channelings with a liquefaction of this medium.

Sch. Pombe is a yeast easily attenuated. Fermentation is very active and manifests itself as top fermentation. The optimum temperature for fermentation is situated between 30° and 35° C. This yeast provokes an apparently strong fermentation of beer wort: it ferments maltose, saccharose, dextrose, levulose, raffinose, and α-methylglucoside. On the other hand, it is able to ferment inuline and dextrine.

SCHIZOSACCHAROMYCES MELLACEI. Jörgensen [2]

This yeast was discovered by Greg [3] from Jamaican molasses used in the manufacture of rum. It has been described by Jörgensen and Holm. It is a species closely related to *Schizosaccharomyces Pombe*

[1] See references under this subject for *Sch. octosporus.*

[2] Jörgensen, A. Die Mikroorganismen der Gärungsindustrie, 5th edition P. Parey, Berlin, 1909.

[3] Greg, P. The Jamaica Yeasts. Bulletin of the Botanical Department, Jamaica, Vol. 2, 1895.

from which it may scarcely be distinguished by its morphological characteristics. The cells have the same shape as those of *Sch. Pombe* but are generally a little larger (Fig. 78).

Developing on carrot slants, Guilliermond found the measurements to be about 9.5 μ by 5.1 μ; the cells of *Sch. Pombe* under the same conditions are about 7 μ long and 4.5 μ wide (Fig. 77).

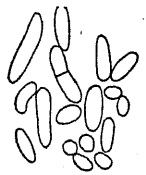


Fig. 77. — Vegetative Cells of *Schizosaccharomyces Mellacei* from Cultures on Slices of Carrot.

Lepeschkin has noticed in this species as in the former one, the appearance of a true mycelium in deposits at the bottom of the culture flask.

Sporulation of this yeast is obtained easily at the end of a few days on beer wort gelatin in hanging drops. The observations of Guilliermond[1] have shown that the ascs result from an isogamic coplation which is accomplished in the same manner as with *Sch. Pombe*. The ascs have a shape like a dumb bell, both swelled portions being connected by a narrow canal (Fig. 78). The ascospores always to the number of four appear in pairs in each large part of the asc. Cases of parthenogenesis may be often observed in which the ascs result without previous copulation. The ascospores are long and rounded (about 4 μ in diameter). They are very refractive, and are clothed with a membrane impregnated with starchy materials which are stained blue by iodin in potassium iodide.

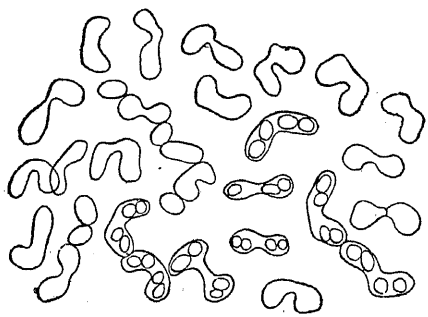


Fig. 78. — Formation of the Asc in *Schizosaccharomyces Mellacei.*

This yeast produces no scum on beer wort but forms a ring at the end of four or five months. Plate cultures in gelatin and streaks on gelatin offer growths which on the surface and in the medium are closely detailed. *Sch. mellacei* ferments beer wort at 25°; there are signs of top fermentation with a broken-up deposit, not very compact. During fermentation it liberates an agreeable odor. It ferments dextrose, maltose, levulose, saccharose, raffinose, d-mannose, dextrine, α-methyl-glucosides and inulin; it is distinguished from *Sch. Pombe* by the fact that it ferments d-mannose on which the latter has no action.

According to Greg this yeast produces several types which are characterized by the peculiar odor given off during fermentation, or

[1] Guilliermond, A. Rem. sur la copulation du *Schizosaccharomyces mellacei.* Bull. Soc. Bot. de Lyon, 1903.

by the amount of alcohol, which varies between 6.6 and 7.6 per cent by volume. These types also differ by the rate at which cellular multiplication takes place.

It seems appropriate to mention a very interesting *Schizosaccharomyces* which we[1] have been able to observe. This was sent by Professor Beijerinck under the name of *Sch. mellacei*. An examination of this yeast shows that it differs from *Sch. mellacei* by a complete disappearance of sexual processes. (Fig. 79.) The ascospores, always to the number of four, are formed in ordinary rectangular or elongated cells without any previous copulation. The vegetative cells are much smaller than those of *Sch. mellacei* or *Sch. Pombe*. On carrot slants their average size is 6.8 μ long and 3.5 μ wide. The ascospores are about the same size (about 4 μ) as those of *Sch. mellacei* and *Sch. Pombe*.

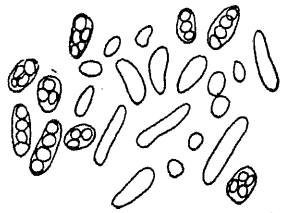


Fig. 79. — Parthenogenetic Variety of *Schizosaccharomyces Mellacei*.

By its morphological characters, it differs from *Sch. mellacei* and *Sch. Pombe*. Unfortunately no study was undertaken of its biochemical features, and we are not able to state whether it is a new species or whether it is a variety of *Sch. mellacei* and *Sch. Pombe* in which sexuality has disappeared.

SCHIZOSACCHAROMYCES ASPORUS. Eykmann

This yeast has been described by Eykmann;[2] it is a yeast used in the manufacture of arrack (the alcoholic drink of Java made from molasses from sugar refineries and rice powder). It is distinguished from *Schizosaccharomyces Pombe* by the fact that it does not produce endospores. Beijerinck thinks that it is an asporogenic variety of *Sch. Pombe*. On nutrient gelatin, it produces white and brown colonies; the white colonies give more ascospores than the brown colonies. It inverts and ferments saccharose.

SCHIZOSACCHAROMYCES APHALARAE CALTHAE. Sulc

This yeast was discovered by Karel Sulc[3] in the larvae of *Aphalarae calthae* (Homoptera). It possesses spherical cells which are

[1] Guilliermond, A. Remarques sur la copulation du Schizosaccharomyces mellacei. Bull. de la Soc. Botanique de Lyon, April, 1903: Thesis for the Doctorate mentioned elsewhere in this volume.

[2] Eykmann, C. Mikrobiologisches über die Arrakfabrikation in Batavia. Cent. Bakt. 16, 1894.

[3] Sulc, K. Pseudovitellius und ähnliche Gewerbe der Homopteren sind wohnstätten symbiötischer Saccharomyceten. Sitzungsberichte der König. Böhm. Gesellsch. der Wessinschaften in Prag. March 30, 1910.

short (about 1 to 2 μ in length) containg a nucleus and metachromatic granules. The cells are often grouped in twos. (Fig. 80, 1 to 4.)

SCHIZOSACCHAROMYCES FORMOSENSIS. Nakazawa [1]

This species was isolated recently by Nakazawa from sugar products in Formosa. On beer wort, the cells are ellipsoidal or irregular

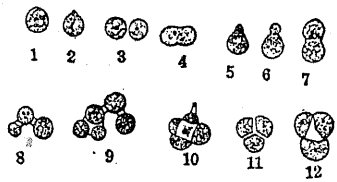


Fig. 80. — *Sch. Aphalarae Calthae.*

1 and 2, Vegetative cells; 3, two gametes about to unite (?); 4, egg resulting from the fusion of the two gametes (?); 5, 6, cells dividing by budding; 7–9, cells dividing by partition; 10–12, beginning of germination of the ascospores (after Sulc).

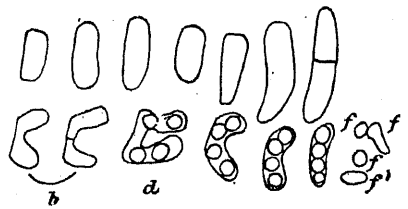


Fig. 80–A. — *Schizosaccharomyces Formosensis*, var. *Tapaniensis.*

b, Copulation; *d*, ascs; *f*, f^1, germination of spores (after Nakazawa).

in shape (9.2–16.8 × 4.8 μ but usually about 7.2 × 6–9 μ). The optimum temperature for budding in beer wort is 32° C. The ascs are formed at the end of 7 days and are derived from an isogamic copulation similar to those in *Schizosaccharomyces Pombe.* They contain ellipsoidal ascospores with no glycogen, but their walls are impregnated with starch. On wort, a ring is formed at 25 to 37° C. and at 32° C., but not below. At 25 to 27° C., a scum is formed. The temperature limits for scum formation are 25 to 27° C. and 37° C. This yeast ferments dextrose, inuline, dextrine, d-mannose, d-galactose, d-fructose, saccharose, maltose, raffinose, and α-methylglucoside.

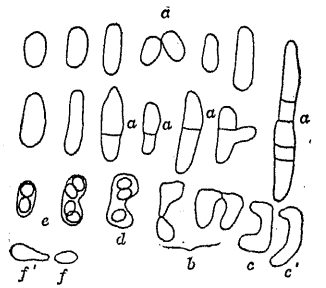


Fig. 80–B.— *Schizosaccharomyces Formosensis.* Vegetative Cells.

Formation of the asc, and germination of spores (after Nakazawa). *a*, vegetative cells; *b*, *c*, c^1, copulation; *e*, *d*, germination of spores.

Nakazawa described two other varieties of this yeast, *Schizosaccharomyces Formosensis* and *Schizosaccharomyces Formensis*, var. *akoensis.* This latter variety differs from the former by larger cells and larger ascospores and also that the ascs are formed at the end of 5 days. No ring is produced at 32° C. and scums are not produced at all. *Schizosaccharomyces Formosensis*, var. *tapaniensis*, another variety,

[1] Nakazawa, R. Ueber Garungsmikroorganismen von Formosa II. Ber. der Inst. für Exper. Forshungen, 1914.

has cells intermediate between the two previous species, forms its ascs at the end of four days and produces a ring at 32° C., but never forms a scum.

SCHIZOSACCHAROMYCES SAUTAWENSIS. Nakazawa

This species was isolated by Nakazawa from sugar in Formosa. It has elliptical cells when grown in beer wort (7.2–8.4 × 4.8 usually, rarely 7.2–19.2 × 4.8 μ). The optimum temperature for growth on beer wort is 32° C. Ascs

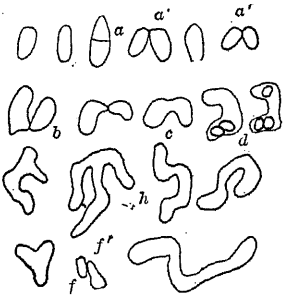


Fig. 80–C. — *Schizosaccharomyces sautawensis.* Vegetative Cells.

b, *c*, and *h*, Copulation; *d*, ascs; *f*, *f*¹, germination of spores (after Nakazawa).

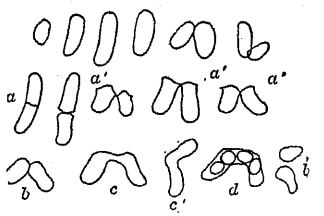


Fig. 80–D.—*Schizosaccharomyces formosensis.*

b, *c*, *c*¹, Copulation of the asc; *f*, germination of spores (after Nakazawa).

are formed at the end of seven days. They are derived from an isogamic copulation, possessing ellipsoidal ascospores without glycogen and with starchy walls (2.5 × 3 to 3.75 μ). A ring is formed at from 25° to 32° C., but none is produced at 37° C. It gives a scum at from 25 to 27° C. It ferments dextrine, dextrose, d-mannose, d-galactose, d-fructose, saccharose, maltose, raffinose and α-methylglucoside.



Fig. 80–E. — *Schizosaccharomyces Nokkoensis.*

a, Vegetative cells; *b*, germinating spores; *g*, ascs (after Nakazawa).

SACCHAROMYCES NOKKOENSIS. Nakazawa

Isolated under the same conditions as the preceding type, this yeast possesses ellipsoidal cells on beer wort (9.6–10.8 × 3.6–5.25, rarely 4.8–16.6 × 3.6–5.2 μ). The optimum temperature for growth in beer wort is 32° C. Ascs are formed after two months, without a copulation. The ascospores are ellipsoidal, spherical, or hemispherical (3 × 5 microns) without glycogen and with starchy walls. A ring is produced on beer wort at 25–32° C.

but more at 37°. No scum is formed. It ferments dextrine, dextrose, d-mannose, d-galactose, d-fructose, saccharose, maltose and raffinose.

SCHIZOSACCHAROMYCES CHERMETIS ABIETIS. Sulc

This yeast was found in *Chermes abietis* and resembles *Sch. Pombe* very much. The cells are oval. They were not cultivated by Sulc. In the larvae of *Psylla Foersteri* he found *Sch. Aphidis; Sch. Psyllae Foersteri* was found in the larvae of various homoptera. He also observed in certain of the homoptera, fungi resembling the *Schizosaccharomyces* in which multiplication was accomplished by division or budding and which did not form ascospores. The author gave them the generic name of *Cicadomyces*. These are distinguished from the *Schizosaccharomyces* by the fact that their division remained for a long time incomplete; the cells remain united at their apexes and are able to form chains of cells. The author describes *C. Ptyeli lineati* and the *C. Aphalarae calthae.*

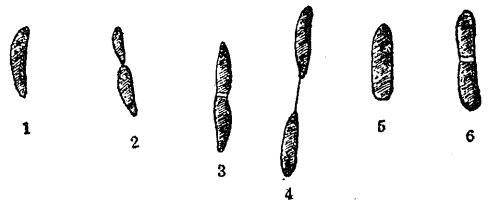


Fig. 81. — *Sch. Chermetis strobilobii* (1 to 4) and *Sch. Chermetis abietis* (5 and 6) (after Sulc).

Hollande [1] has observed the yeast forms in the blood of other insects. He studied the blood of the locust (*Caloptenus italicus*). Under normal conditions, the blood of this insect is yellow, but when it is infected with the yeast it is a milky white. Hollande could reproduce the infection only by injecting blood from an infected insect into a healthy insect. These insects died i from 5 to 7 days and their blood was found to be filled wit the yeast parasite.

The yeast structures were cylindrical. Their dimensions varied from 4.98 to 6.64 microns in length and from 1.70 to 2 microns in width. A vacuole is present in each end of the cell. Buds may appear at the end. After staining with ferric hematoxyline a circular nucleus is observed which is rich in chromatin. This parasite grows well on blood serum with a white scum; on gelatin, growth is abundant. Fine filamentous structures may be seen at the edge of the cells. No spores could be demonstrated.

Second Group

Yeasts multiplying by budding, and in which the ascs are derived from a copulation, or show traces of sexuality in their origin.

[1] Hollande, A. Ch. Formes levures pathogènes observée dans le sang d'Acridium (Caloptenus italicus). Comp. Rend. Acad. Sci. 168 (1919), 1341–1344.

Genus II. Zygosaccharomyces. Barker

Ascs resulting from a copulation of two cells. Ascospores in a membrane which is smooth.

ZYGOSACCHAROMYCES BARKERI. (Barker) Saccardo-Sydow[1]

This species was found by Barker[2] in ginger beer to which had been added saccharose and nutrient salts. They have the shapes of small oval cells. (Fig. 82.) The maximum temperature for budding on nutrient gelatin is in the vicinity of 37–38° C.; the minimum near 10–13° C.

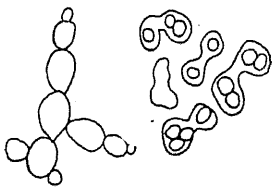


Fig. 82. — *Zygosaccharomyces Barkeri.* Vegetative Cells and Ascs (after Barker).

The ascospores appear easily, not only on plaster blocks but in great numbers on other media (nutrient gelatin, damp bread, potato, carrot). The maximum temperature for the formation of ascospores on plaster blocks is 37–38° C., the minimum around 13°. At 25–27° the first rudiments of ascospores appear at the end of 20 to 24 hours.

The ascs result from an isogamic copulation between two cells. This copulation, which has been described by Barker, is accomplished in the same manner as in *Sch. Pombe* and *mellacei*. Two cells identical in characteristics, unite by means of a copulation canal formed by the fusion of a little projection from each cell. The fusion remains incomplete and the cells look like a dumb-bell. The ascospores are formed in the swelled portions of the asc. Their number varies between two and four. (Fig. 82.) *Zygosaccharomyces Barkeri* does not form a scum on sugar solutions, but at the end of 10 to 14 days a ring, made up of oval cells, appears. It ferments dextrose, levulose, saccharose, and α-methylglucosides but neither maltose, lactose nor dextrine.

ZYGOSACCHAROMYCES PRIORIANUS. Klöcker[3]

This species, recovered by Klöcker from the bodies of bees, possesses cells of varying shapes, elongated, round or oval, sometimes in the shape of a sausage and almost always united. The temperature limits of budding are: maximum, 36–38° C., and minimum, 3–8° C.

Spores are easily produced on gelatin or wort, carrot or agar. On plaster blocks, on the contrary, they form very slowly. The limits for the formation of ascospores on plaster blocks saturated with beer wort, are 27–28° and 3–9° C.

[1] Saccardo and Sydow. Syllage fungorum, Vol. II, 1902.

[2] Barker, P. A conjugating yeast (Zygosaccharomyces n. gen.). Proc. of the Royal Society, Vol. 6, July 8, 1901. On the spore formation among the Saccharomycetes. Journal of the Federate Institutes of Brewing, 8, 1902.

[3] Klöcker. In Lafar's Handbuch der technischen Mykologie, Jena, 1904–1905.

Klöcker showed in 1904 that the ascs of this yeast are derived from an isogamic copulation; consequently it is related to the genus *Zygosaccharomyces*. Copulation is accomplished exactly as in *Zy. Barkeri*. The ascs are formed of two large portions united by a canal (Fig. 83). The ascospores in the number of 2 or 4 are formed in each large part of the asc; they are round or oval. We have shown that quite a few of the cells form ascospores without having undergone copulation. Parthenogenesis is, then, rather frequent. (Fig. 83, a, b, c.) This yeast forms a rather scant scum but very often a well-developed ring. The appearance of the colonies on gelatin at the temperature of the laboratory resembles the shape a cupule. This yeast ferments dextrose, levulose, saccharose and maltose but not lactose.

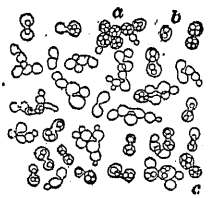


Fig. 83. — Formation of the Asc in *Zyg. Priorianus*.

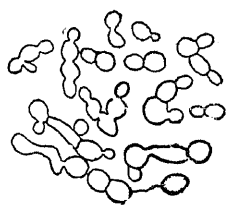
ZYGOSACCHAROMYCES JAVANICUS. de Kruyff

This species was isolated in Java by de Kruyff [1] in 1908 on partly decomposed foliage. It is a yeast with elliptical cells from 4 to 8 μ in diameter. The optimum temperature for budding is situated between 34 and 35° C.; the maximum is around 38°. Spores appear easily on gelatin and are preceded by an isogamic copulation. *Zy. javanicus* does not form a scum. It is a bottom yeast which ferments dextrose, levulose, saccharose and d-galactose.

ZYGOSACCHAROMYCES JAPONICUS. Saito

Syn.: Soya-Kahmhefe Saito

This yeast was discovered by Saito [2] in 1906 among the products of fermentation of Soya; it was, at first, given the provisional name of Soya-Kahmhefe. It is a yeast with round or oval cells (Fig. 84) which frequently gives long threads made up of budding units. The ascs in certain numbers are observed in the scum growing on Koji or in cells developing on gelatin. They are easily obtained on Gorodkowa's gelatin medium. Saito in 1909 showed that they were preceded by a copulation similar to that of *Zyg. Barkeri* and on account of this, this yeast is attached to the *Zygosaccharomyces*. The ascs resemble retorts united


Fig. 84. — *Zyg. Japonicus* (after Saito).

[1] de Kruyff, E. Untersuchungen über auf Java einheimische Hefenarten. Cent. Bakt. 21, 1908.

[2] Saito, K. Mikrobiologische Studien über Soyabereitung. Cent. Bakt. 17, 1906.

by their necks. (Fig. 85.) The number of ascospores varies in each asc. They are spherical (2.7 to 6.3 μ in diameter) and are relatively resistant. Germination is accomplished by budding. Guilliermond has noticed frequently cases of parthenogenesis in this yeast in which the ascs form without preliminary copulation. On decoction of "Koji," this yeast produces a white farinaceous scum. On the surface of this scum a number of bubbles of carbon dioxide form. The scum increases slowly and turns to a light brown.

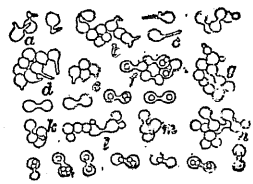


Fig. 85.— Copulation and Formation of the Ascs in *Zyg. Japonicus.*

The giant colonies are raised, dry and light gray in color. The surface is concentrically ringed and cut up by fissures. The edge of the colony is notched. Under the colony numerous bubbles of gas may be found.

The cultures on gelatin have a light brown appearance. This species ferments dextrose, levulose and maltose but has no action on δ-galactose, lactose, saccharose, melibiose, raffinose, α-methyl-glucoside and inuline. *Zyg. japonicus* by the character of its scum, approaches the genera *Willia* and *Pichia* very much. It is distinguished, however, by the fact that the scum completely developes only in decoctions of "Koji." Takahashi and Yukawa[1] have recently redescribed this yeast as follows:

This species was isolated from many samples. Since this yeast and *Zygosaccharomyces salsus* develop and form particular grayish brown films even on a "Shoju" which any other kinds of film-forming yeast could not grow, both these yeasts are feared in storing "Shoju." Moreover, this species very easily forms large numbers of sporulated cells.

Young cells from the surface cultures on "Koji"-extract agar are round (commonly 4–8 c.c.) or oval, and contain glycogen. In old cultures club-shaped or mycelial cells are often observed. Most of the cells in a diluted "Shoju" are elongated abnormally and increase the number of vacuoles.

On plate culture of wort gelatin, this yeast forms a grayish white, crater-like, elevated colony with smooth periphery, and the color turns brownish after the lapse of time. On "Koji"-extract-gelatin streak culture, the growth shows a grayish white, somewhat dried, lustered, folded covering with fine toothed edge. On "Koji"-extract culture at 23° C. it forms mealy white, small filmy fragments on the surface, and covers the whole surface after 3 days. The film

[1] Takahashi, and Yukawa, M. On the budding fungi of "Shoju-Moromi." Original Communications of the 8th International Congress of Applied Chemistry, 14 (1912), 155–171.

crinkles like crepe paper, increasing its thickness, and its color changes to yellowish brown. After three weeks the film gradually falls down and deposits a great deal of sediment on the bottom, leaving a thin film over the surface, and at last only a few parts of the yeast ring remain along the wall. In wort culture at 23° C., after keeping the culture for seven days film does not form, although gas bubbles ascend through the medium. After three months a well-defined yeast ring and thin film become observable, but this film has never been folded at all. This species reproduces and forms its particular film even in "Koji"-extract or "Shoju" which contains 23% of NaCl.

It is most noticeable that this species forms a grayish brown, folded film on the surface of sterilized "Shoju" after a long time, while other races which we isolated from "Shoju-Moromi" cease the reproduction of their cells in the same medium.

This species ferments dextrose, maltose, levulose, but does not ferment saccharose, lactose, raffinose, α-methylglucoside, galactose.

It rarely produces spores in a yeast ring of "Koji"-extract cultures after three months. After Gorodkowa's method, sporulation occurs often after 10 days at 28° C. Following the diluted "Shoju" method which has been described in the preceding plate, a large number of sporulated cells occur in the yeast ring after 4–5 days. The spore is transparent with a somewhat thick wall; it is round or oval in shape, 2.5–6 μ in size, and contains a few tiny grains. The processes of formation and germination of spores in this yeast and other relations are similar to the preceding yeasts. This yeast seems to be identical with *Zygosaccharomyces japonicus*, Saito.

Mitsuda and Nishimura have found in the fermentation of Soya an asporogenic yeast. Kita has recently described a yeast similar to *Zygosaccharomyces major* (Takahashi and Yukawa) which differs in that galactose is not fermented.

ZYGOSACCHAROMYCES SOYA? (Saito) Guilliermond

This yeast was found with the preceding yeasts by Saito[1] in the products from the preparation of Soya sauce and designated as *Saccharomyces Soya*. It is made up of spherical or oval cells (4 to 8 μ average size) with relatively resistant walls and homogeneous contents with large vacuoles. (Fig. 86.) No ascospores are produced on plaster blocks, or in yeast water to which dextrose has been added or pure agar; abundant sporulation is observed, however, in the rings formed on a decoction of Koji. The asci are usually made up of two

[1] Saito, K. Mikrobiol. Studien über die Soya-Bereitung. Cent. Bakt. 17, 1906.

swelled parts connected by a canal and offer a resemblance to the ascs of the *Zygosaccharomyces* and we shall not hesitate to attach this yeast to this genus although copulation has not been observed up to the present time. (Fig. 86.)

The ascospores are generally to the number of from 1 to 4 per asc and are located in each part of it. They are spherical, hyaline and possess a resistant wall. Their diameter is from 2.7 to 4.5 microns.

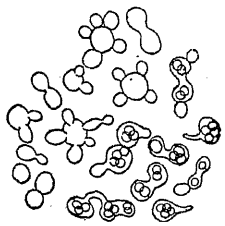


Fig. 86. — Vegetative Cells and Ascs of *Zygosaccharomyces Soya* (after Saito).

Giant colonies are a grayish brown color; they are raised in the center and have a greatly notched edge. On plates, colonies are produced which resemble dots, round and moist. On gelatin streaks or stabs the yeast offers a greenish coloration along the line of inoculation and on the surface a grayish yellow. *Zygosaccharomyces soya* ferments dextrose, levulose, d-mannose, d-galactose and maltose easily. It does not act on saccharose, inuline, α-methylglucoside, lactose, melibiose, or raffinose. It inverts saccharose but does not ferment it. Its invertase does not pass through the cell wall and is, therefore, an endoenzyme.

ZYGOSACCHAROMYCES LACTIS α. W. Dombrowski

This species isolated by Professor Jensen from beer has been described by Dombrowski [1] (1910). On beer wort the cells are spherical and have an average diameter of 4.7 μ. Sporulation is preceded by an isogamic copulation which is effected after the normal procedure. The ascs which result are made up of two enlarged portions connected by a canal. (Fig. 87, 2 and 3.) The ascospores are formed in these two enlarged parts and vary from one to four in each asc. Germination is accomplished by an absorption of the wall of the asc after which ordinary budding is accomplished. This species produces, on beer wort at room temperature, a scant scum made up of cells with a normal shape.

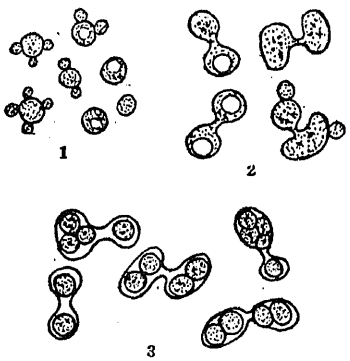


Fig. 87. — *Zyg. Lactis* α.
1. Vegetative cells. — 2 and 3. Ascs (after Dombrowski).

The colonies on plates are lenticular and are round or torpedo shaped. In stab cultures the growth extends about 3.5 centimeters below the surface. Giant colonies offer a crater-

[1] Dombrowski, W. Die Hefen in Milch und Milchprodukten. Cent. Bakt. 28, 1910.

iform center about which there is a sort of raised wall. The edge is slightly fringed and partly covered with ridges. It produces an energetic fermentation in beer wort which it clears in about 10 days. The wort is slightly colored and made to give off an aromatic odor. In about 100 c.c. of wort, after five and a half months, it forms about 4.5 grams of alcohol. *Zygosaccharomyces lactis* α produces an active fermentation in milk. It ferments lactose, saccharose and dextrose and d-galactose but has no action on maltose. Along with the alcohol and carbon dioxide which it produces, small quantities of acids are produced.

ZYGOSACCHAROMYCES BAILII (?) (Lindner) Guilliermond

Syn. SACCHAROMYCES BAILII. Lindner

This yeast was isolated from beer by Lindner.[1] The cells are large and elongated, and have resistant walls. The contents are ordinarily homogeneous and brilliant. In old cultures they possess a peculiar irregular ameboid shape.

At the end of a long series of cultures on gelatin, this yeast loses its property of sporulating. The ascospores are very refractive almost devoid of granules and resistant walls. A copulation seems to be indicated by the shape of the asc which is made up of two enlarged parts. (Fig. 88.) Then it is about certain that this species may be incorporated in the genus *Zygosaccharomyces*.

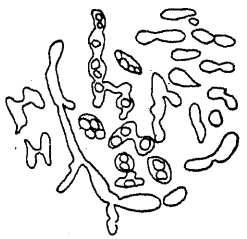


Fig. 88. — *Saccharomyces Bailii* (according to Lindner).

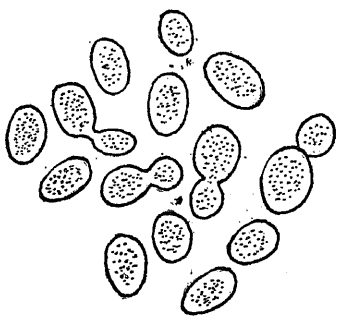


Fig. 88–A. — *Zygosaccharomyces Mandshuricus*. Sporogenic Race from Sediment in Beer Wort at 28° C. (after Saito).

This opinion is confirmed by the presence of ameboid cells in old cultures, some of which sporulate and which seem to be endowed with unfruitful attempts at copulation. No scum is formed by this yeast; on hop wort, it develops at the bottom of the culture. Fermentation is very feeble and growth less abundant. The must gives off an aromatic odor.

Giant colonies on gelatin or beer wort have a slow growth and remain small. The surface is glistening and a grayish white. Gelatin is not liquefied. The cells often present an ameboid shape.

[1] Lindner, P. S. farinosus et Bailii. Woch. Brau. 1893.

On gelatin streaks, a whitish deposit forms. In gelatin stabs the yeast shows a tendency to form lateral rays. In gelatin to which must has been added, vesicles of carbon dioxide are formed here and there. This yeast is able to ferment only dextrose and levulose.

ZYGOSACCHAROMYCES MANDSHURICUS. Saito[1]

This yeast was isolated from Chinese yeast which is used to prepare the "l'eau de vie" in Manchuria, an alcoholic drink known as Sorgho. The cells are round or oval (6.5–9.5 μ in diameter). The giant colonies are round or oval with a flat edge. On plaster blocks

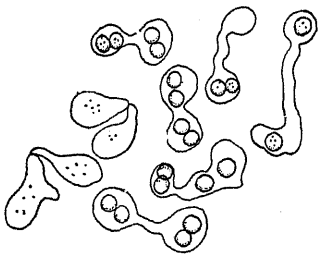


Fig. 88–B. — *Zygosaccharomyces Mandshuricus* (after Saito). Copulation and Formation of the Ascs.

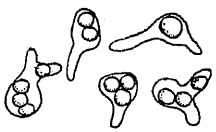


Fig. 88–C. — *Zygosaccharomyces Mandshuricus*. Parthenogenetic Ascs from Cells of a White Race (after Saito).

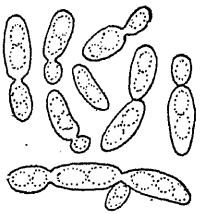


Fig. 88–D. — *Zygosaccharomyces Mandshuricus*. Asporogenic Variety. Cells from Sedimental Growth in Wort at 20° C. (after Saito).

in beer wort, and on Gorodkowa's medium, ascs are formed containing one to four spores (4–5 μ in diameter). These result from an isogamic copulation. The temperature limits for sporulation are 30 to 35° C. This yeast ferments dextrose, levulose, and mannose energetically, saccharose feebly.

ZYGOSACCHAROMYCES MELLIS ACIDI. Richter

Isolated from honey undergoing an alcoholic fermentation, this yeast possesses cells of small dimensions. The ascs are formed by isogamic copulation. The yeast ferments glucose, fructose and saccharose actively and galactose feebly, but has no action on other sugars.

[1] Saito, K. Mikrobiologische Studien über die Bereitung des Mandschurischen Branntweins. Report of the Central Laboratory, South Manchuria Railway Co. 1914. Bull. Past. Inst. 12 (1915) 1.

ZYGOSACCHAROMYCES NADSONII. Guilliermond

This species was isolated by Guilliermond [1] from a large bottle of orange syrup at Hospital 101 at Lyon during the summer of 1915. In this it caused a very active fermentation. After 12 hours in beer wort at 25–30° C., this yeast forms a white deposit at the bottom of the flask. In a few days there is produced over the surface of the liquid a very delicate covering. Later a ring develops which falls to the bottom of the flask when it is disturbed. This ring does not seem to re-form. The cells are generally round or oval in beer wort cultures which are 12 hours old. Sometimes they are isolated but generally they adhere in a small group; after 24 hours this tendency is greatly increased. The cells are generally round or oval and form buds around their peripheries. The cells may resemble those of *Torula*. After 8 to 14 hours the elongated cells may become rather numerous. On must agar, the colonies develop quickly; on carrot, a white blanket growth is formed.

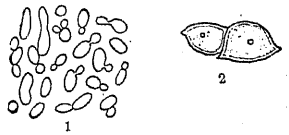


Fig. 88–E. — *Zygosaccharomyces chevalieri*. 1, Cells from Sediment in Beer Wort 24 Hours at 25° C.; 2, Ascospores.

The minimum temperature seems to be situated between 3 and 5° C. At 5° C. and up to 15° C. the yeast develops slowly. At 18° C. and 20° C. its development becomes more rapid. The optimum temperature is situated near 30° C. and the maximum is between 41° C. and 42° C. In the vicinity of these temperatures the yeast shows a tendency to elongate and form cells like the *Pastorianus* type.

The ascospores form very abundantly after a few days on Gorodkowa's media. They seem to be formed in great numbers but less rapidly on carrot and beer wort agar. The ascs result almost universally from a heterogamic conjugation. It is very easy to follow all the stages in this phenomena in a culture on Gorodkowa's agar. At the moment when conjugation is about to occur most of the cells affected show in their center a large number of small fat globules. Almost all are united in small colonies made up of a small number of cells. Conjugation is effected between a mother cell and an incompletely developed daughter cell. The mother cell plays the rôle of a female gamete while the daughter cell represents the male gamete. The gametes are then represented by cells of different ages. The male gamete (microgamete) is a cell which has not achieved its full growth, while the female gamete (macrogamete) is full grown.

[1] Guilliermond, A. Zygosaccharomyces Nadsonii. Nouvelle espèce de levures à conjugaison hétérogamique. Bull. Soc. de France, 34 (1918).

The two gametes unite by a copulation canal formed by the union of two small projections sent out from each cell. During this phenomenon the small cell remains adherent to the mother cell and after the development of the copulation canal may detach from this cell. The contents of the male gamete emigrate to the female gamete, both protoplasms fuse, and the female gamete is transformed into an egg; this changes very quickly into an asc, and each asc contains usually from one to two ascospores; exceptionally one may find three, or even four.

Besides this heterogamic conjugation which has been described, frequently one may observe a series of transitional forms between the iso- and heterogamic. The conjugation presents the same characteristics in cultures on carrot and beer wort. However, in the latter medium the yeast seems to take on the elongated form.

The minimum temperature for sporulation on Gorodkowa's agar seems to be situated around 5°. At this temperature the first rudiments of ascospores appear at the end of two weeks; at from 13–15° the ascospores form at the end of eight days; from 19–20° they appear at the end of 56 hours; the optimum temperature seems to rest between 23 and 30°, while the maximum temperature is situated somewhere between 30 and 32°. The ascospores remain enclosed in the wall of the asc. During the first stages in germination, the wall is ruptured, and the ascospores germinate by ordinary budding.

In plate cultures the colony is of yellowish white with a peripheral zone made up of canals; the center is a little elevated and is constructed of rather thick, irregular folds. In streak cultures the colony presents the same characteristics. In stab cultures the yeast develops abundantly along the line of inoculation and forms a large number of small colonies. The colonies enlarge toward the surface. The giant colonies on must gelatin at from 15–20° have a dry appearance and are only a few millimeters in diameter. The color is yellowish white, the center is slightly raised.

This yeast inverts saccharose when cultivated in must. It forms carbon dioxide which gives evidence of fermentation. This fermentation is well demonstrated by Lindner's droplet culture method. According to this method the yeast ferments dextrose and levulose. It has no action on lactose, d-galactose, maltose, dextrin and raffinose.

The existence of a heterogamic conjugation is of special biological interest, because heterogamy up to the present time, has been rarely observed among yeasts. Guilliermond found it in the yeasts which he named *Saccharomyces chevalieri*. In this same paper Guilliermond has given a rather extended discussion on this subject in its relations to the yeasts.

ZYGOSACCHAROMYCES MAJOR. Takahashi and M. Yukawa [1]

This yeast was isolated from samples taken at different stages during the ripening of " Shoju."

In "Koji "-extract or wort culture after 4 days at 20° C., the cells are mainly spherical (3.7–7.5 μ), sometimes oval, their contents are homogeneous, and sometimes exhibit vacuoles. The glycogen reaction is evident in every cell. Cells in yeast ring of " Koji "-extract culture after 20 days at 20° C. are so irregular that a cell may be as small as 2.5 microns and as large as 10 microns. The occurrence of these cells seems to be somewhat prolonged in wort or " Koji " extract containing a quantity of salt. Old culture in the same media after 2–6 months at room temperature exhibits not only the cells which are similar to Will's film cells of the first generation, and permanent cells, but also very highly elongated, mycelial ones.

In " Koji "-extract or wort gelatine plate after 7 days at room temperature this species forms grayish white, round, bright, waxy colonies. On " Koji "-extract-agar plate after 30 days at 27° C. it grows with somewhat brownish, waxy, dull lustered, elevated covering. Margin shows somewhat paralleled streamy canals. On glucose-sake-agar after 10 days at 25° C. it forms a grayish white, waxy covering with slightly elevated sides. The central part is somewhat concaved and the marginal part dull toothed. On " Koji "-extract gelatin stab after 30 days at 15° C. it forms waxy, feeble lustered, brownish, elevated isles at the mouth of the stab canal, and rosary-like colonies with gas bubbles along the canal. This species grows in many fluid media, and according to the appearance of its fermentation it belongs to the so-called bottom yeasts. In " Koji "-extract culture at 25° C. a yeast ring appears first after 3 days, but it does not form any complete ring even after 6 months, while the sedimental yeast crop becomes somewhat plentiful after 3 weeks. Its development in wort or hopped wort seems to be inferior to that in "Koji " extract. Its resisting power against NaCl is so striking that it can grow tolerably in " Koji " extract or wort containing 20% NaCl.

According to Lindner's method this species ferments dextrose, levulose, mannose, saccharose, maltose, but does not ferment galactose, lactose, raffinose, α-methyl-glucoside.

This species is one of easily sporulable kind among all the *Zygosaccharomyces* isolated from " Shoju-Moromi." This yeast does not form spores on gypsum-block at all. Sporulated cells occur very rarely in yeast ring developed in " Koji "-extract culture after 3 to 6

[1] Takahashi, and Yukawa, M. Original communications, Eighth Internatl. Congress of Applied Chemistry, V. XIV, 1912, p. 162.

months at 20° C. or on Gorodkowa's agar after 20 days at 25° C. On the other hand, following the diluted "Shoju" culture which has been described above large numbers of ascs easily occur in the yeast ring within 7–15 days. The processes of formation and germination of spores are similar to those of *Zygosaccharomyces soja* which have already been described. Spores are transparent, round or oval, and commonly 3–4.5 μ in diameter. A few tiny grains are contained in each spore. The total number of spores in each asc is 1–4; but the number of spores which occurs in each part is quite variable.

This species seems to be nearly similar to *Torula "Shoju"* var. *minuta* which was isolated from "Shoju-Moromi" by J. Nichimura. It is necessary to ascertain the sporulation of the latter yeast after our method.

This yeast differs distinctly from *Zygosaccharomyces soja* and asporogenic species of *Zygosaccharomyces* by the following characteristics: This species ferments saccharose, and the time required for sporulation of this yeast is far shorter, and the number of sporogenic cells in yeast ring is always abundant.

Zygosaccharomyces salsus distinguishes itself from this yeast by the formation of a particular film.

ZYGOSACCHAROMYCES CHEVALIERI. Guilliermond

This yeast was isolated from products of fermentation made in Occidental Africa by the inhabitants for alcoholic drinks. They were secured through the Chevalier Mission. On beer wort at 25° C., there is formed at the end of 24 hours an abundant sedimental deposit at the bottom of the culture flask and a scum not containing air but with a grayish and slightly viscous appearance. It is very delicate and falls to the bottom of the flask when it is disturbed. Another soon re-forms. The cells in the sedimental deposit are variable in shape, sometimes spherical, usually oval or ellipsoidal. Others are elongated. Their dimensions vary between 2–6 microns long and 4–8 microns wide. Their contents are transparent with a vacuole and many brilliant granules. The cells are generally isolated or united two by two. At the end of 15 days and up to a month, the cells in the sediment show a tendency to elongate and remain united in chains. One may find 5 to 10 elongated cells with lateral buds and branches which make up a sort of pseudomycelium. The temperature limits for budding in beer wort are: maximum, 42–43° C.; minimum, below 5° C. Near these temperatures this yeast does not develop in the form of a sediment nor does it produce a scum. The cells have the same shape as at other temperatures. This yeast sporulates easily

and rapidly on most solid media, such as slices of carrot, gelatin of Gorodkowa, must agar and gelatin. Spores are also formed in scums developed on beer wort. This sporulation is preceded by a heterogamic copulation which has been described before.[1] This copulation takes place between two gametes of variable dimensions and of different ages. One, the female gamete or macrogamete is an adult cell, and very large; the other, the male gamete, or microgamete, is very small, and young, generally a bud being detached from a mother

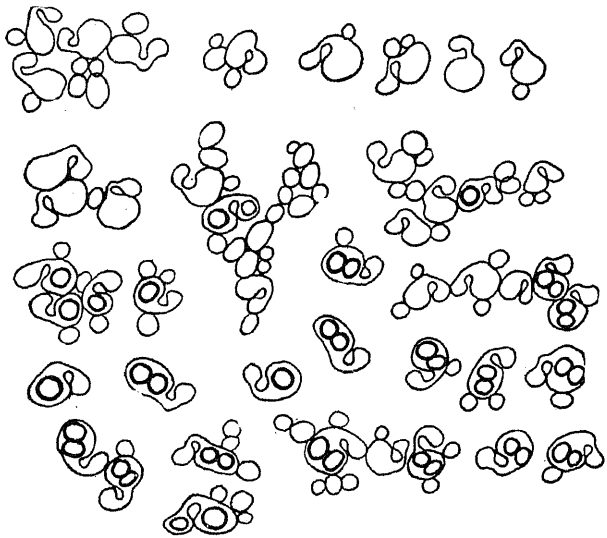


Fig. 88–F. — Heterogamic Copulation in *Zygosaccharomyces Nadsonia.*

cell. The two gametes unite by a copulation canal, then the contents of the microgamete pass into the macrogamete which becomes the egg. A wall is formed across the copulation canal which separates the egg which soon appears as a separate cell. This changes into an asc with from 1 to 4 ascospores, rarely more. By their form, the spores are intermediate between those of the genus *Pichia* and of the genus *Willia* but they approach those of *Pichia* most closely. Their dimensions vary between 1.5 and 2.5 μ.

The temperature limits for sporulation are: maximum, 37–38° C. and the minimum, 8–10° C. The optimum is situated near 25° C. to 30 C. The spores form in from 18 to 20 hours at this temperature. Germination of the spores is accomplished by ordinary budding. The spores enlarge and lose their hemispherical shape when budding.

[1] Guilliermond, A. Sur un example de copulation hétérogamique observé chez une Levure. Comp. Rend. Soc. Biol. 1911.

The giant colony on must agar at 25° C. after 15 days, is well developed. The color is gray, slightly yellow with a dry appearance. The center is folded. The periphery is thin, transparent, and possesses a border made up of fine canals with deep hollows. At the end of two months, the colony possesses a grayish yellow color with a flat dry appearance. The yeast produces no fermentation of beer wort. It inverts saccharose but yields no indication of fermentation in saccharose, dextrose, levulose, maltose, d-mannose, lactose, d-galactose and dextrine.

The presence of a scum causes this yeast to form a sort of connection between Hansen's 1st and 2nd group. However, by its giant colony, its vegetation in liquid media and the shape of its spores, it resembles the genera *Willia* and *Pichia*.

ZYGOSACCHAROMYCES SALSUS. Takahashi and Yukawa, M.[1]

This species was discovered in samples taken from all the factories at Tatsuna. The young cells from the surface culture on "Koji"-extract agar are mostly round (4–8 μ) and rarely oval. The contents are homogeneous and sometimes exhibit vacuoles.

On streak culture at 27° C., the growth shows a grayish white, feeble, finely folded covering. On glucose-sake-agar after 10 days at 25° C. it forms a grayish yellow, folded, elevated covering with streamy margin. On "Koji"-extract culture at 23° C., it forms a few parts of yeast ring without clouding the fluid after three days. The ring gradually grows and increases its thickness. After three weeks a thin film covers the surface. The culture medium which was kept for three months was strikingly decolorized.

Wort culture is similar to the former culture, but this yeast forms a grayish white, folded, thick film on "Shoju" or "Koji" extract which contains a quantity of NaCl. This yeast is easily distinguished from *Zygosaccharomyces japonicus* by this characteristic point.

This species ferments dextrose, levulose and maltose, but does not ferment galactose, lactose, saccharose, raffinose, α-methyl-glucoside.

In formation and germination of spores this yeast is similar to *Zygosaccharomyces japonicus*, but the time required for sporulation of this yeast is longer than that of the former species.

This yeast forms a thick film in some nutrient fluids which contain a quantity of NaCl, but not in the absence of NaCl. More-

[1] Takahashi, and Yukawa, M. Original communications, Eighth Internatl. Congress of Applied Chemistry, v. XIV, 1912, p. 167.

over, this yeast is easily distinguished from the former species by the cell forms, and the time limit of sporulation.

Torula soja (G. and H. Nishimura) seem to be identical with this species. From above differentiation we gave it the name of *Zygosaccharomyces salsus.*

ASPOROGENIC SPECIES OF ZYGOSACCHAROMYCES

Takahashi and M. Yukawa [1]

On the mycological relations this yeast is closely similar to *Zygosaccharomyces soja.* It forms a well-defined yeast ring in "Shoju" and "Koji" extract, but the sporulated cells have never occurred in any yeast ring in spite of the presence of a number of dumb-bell-shaped cells.

According to this, this yeast seems to be a variety of *Zygosaccharomyces soja* which has lost the capacity of forming spores. Subsequently we have continued to cultivate this yeast in various nutrient media for restoring the power of sporogenation. Whether this yeast has lost the faculty of producing spores, temporarily or permanently, has not been determined.

YEAST F. Pearce and Barker

This yeast which belongs to the genus *Zygosaccharomyces* was discovered by Pearce and Barker [2] in 1908 in cider. It possesses oval cells (6.8 by 3.4 μ). The maximum temperature for budding is situated between 30° and 32.5° C. Sporulation is easily accomplished on porous porcelain, on potato or on wort gelatin. The asc which results is composed of two enlarged parts united by a canal. (Fig. 89, *B.*) The ascospores normally to the number of four are situated two in each enlarged portion of the asc. Under exceptional circumstances we might find one ascospore in one enlargement and three in the other. Germination is accomplished by a swelling of the ascospore and a rupture of the wall of the asc; this is followed by normal

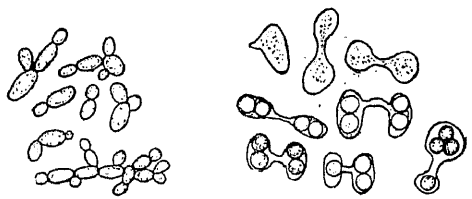


Fig. 89. — Yeasts F. *A*, Vegetative Cells; *B*, Ascs (after Pearce and Barker).

[1] Takahashi, and Yukawa, M. Original communications eighth internatl. Congress of Applied Chemistry, v. XIV, 1912, p. 167.

[2] Pearce, B. and Barker, P. The yeast flora of bottled ciders. The Journal of Agricultural Science, 3, 1908.

budding. Colonies on gelatin plates are spherical with a solid appearance. In streak culture, the growth is slightly humid and milky. This yeast ferments levulose, dextrose, and saccharose.

YEAST G. Pearce and Barker

This species, found in the same environment as the former yeast, generally has oval cells. The maximum temperature for budding is around 32.5° C. (Fig. 90.) Sporulation has been obtained on porous porcelain; it is preceded by a process which seems to be intermediary between iso- and heterogamy. The ascospores are formed in one of the enlarged portions of the asc. (Fig. 23.) Germination is accomplished as in Yeast F. This species develops rather rapidly on beer wort and in all sugar solutions with a scum, in which the cells possess the same shape as those in the deposit. Colonies on beer-wort gelatin are dry, spherical and shriveled. In streaks, the growth is slightly bunched. This yeast produces no fermentation.



Fig. 90. — Zygosaccharomyces G (after Pearce and Barker).

ZYGOSACCHAROMYCES BISPORUS. Anderson [1]

Morphology. In young liquid cultures the cells are oval or ovate; in old cultures they assume various forms with numerous conjugating, but usually no sporulating cells. Elongated cells are common, but there is no mycelial formation. Budding occurs from end or side. The size is 4×6.5 microns. Spore formation occurs on carrot slants at room temperature. Conjugation is most common previous to spore formation, but parthenogenesis is not rare. There are 2–4 ascospores, most commonly 2.

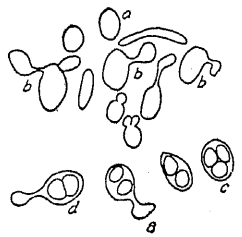


Fig. 90–A. — *Zygosaccharomyces bisporus*, Anderson.

a, Typical cells from Old Agar Slant; *b*, Asporogenic Conjugating Cells; *c*, Ascospore Development without Conjugation; *d*, Ascospore Development resulting from Conjugation (after Anderson).

Cultural Characters. On glucose agar the growth is spreading, dull, flat, and white; later it becomes brownish with small, scattered, wart-like prominences and more glistening surface. There is a filiform growth in gelatin stab and liquefication in beer-wort gelatin in 3 weeks. Pellicle is present on beer wort and some sugar mediums.

Physiologic Properties. It does not ferment glucose, sucrose, levulose, maltose, galactose, or raffinose. No decided change in acidity

[1] Anderson, H. W. Yeast-like fungi of the human intestinal tract. Jour. Infectious Diseases, 21 (1917) 341–386.

occurs in these mediums. There is no change in litmus milk. The culture was isolated from human feces.

Genus III. Debaromyces. Klöcker

Ascs derived by copulation. Ascospores in a single membrane, the surface of which is covered with little elevations

DEBAROMYCES GLOBOSUS. Klöcker

This species was discovered in 1909 by Klöcker [1] in samples of soil from the island of Saint Thomas. On beer wort at 25° C., the cells are spherical (4.5 to 5 microns in diameter). The limits of temperature for budding on beer wort are: maximum, 41.5 to 43; minimum, 5 to 8° C. The ascs develop abundantly on plaster blocks at 32° C. The limits of temperature for the formation of ascospores are: maximum, 34 to 36° C., minimum, 14° C.

From the researches of Guilliermond,[2] it is evident that the ascs result from a copulation. In about 25 per cent of the cases, this copulation is by isogamy between two cells which are more or less closely situated. (Fig. 91.) In all of the other cases copulation occurs between an adult cell and a little bud which was formed by this cell, but which remained attached to it (Fig. 28 c and 91). It is then heterogamic. The yeast may then be considered as a form in which heterogamy is in the process of installing itself. Finally, parthenogenesis is very frequent, more than in the *Zygosaccharomyces*.

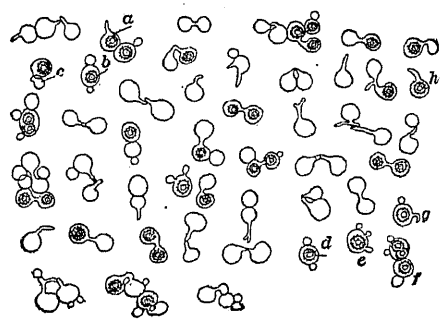


Fig. 91. — Copulation and Formation of the Ascs in *Debaromyces globosus*.

The number of ascospores varies from one to two in each asc, one being more frequent. The ascospores are globular (2 to 3.5 μ). Their surfaces present small elevations. In the center, a small globule of fat is found.

[1] Klöcker, A. Deux nouvelles genres de la famille des Saccharomyces. Comp. Rend. des trav. du lab. de Carlsberg, 8, 1909.

[2] Guilliermond, A. Sur un curieux exemple de parthenogenese observe dans une levure. Comp. Rend. Soc. Biol. 69, 1910. Quelques remarques sur la sexualite des levures. Annales mycologici, 8, 1910; Sur la reproduction de *Debaromyces globosus* et sur quelques phénomènones de rétrogradation de la sexualite observés chez les levures. Comp. Rend. Soc. Biol. 151, 1911.

During germination the ascospores swell up and the warts on their surfaces disappear. Germination is accomplished by ordinary budding. (Fig. 40.) In wort cultures no scum is formed but often a rudimentary ring may be observed. Giant colonies on gelatin, at the end of a month, have a grayish white color resembling wax. The border is almost entire; the center is slightly raised and of a white color. This species ferments dextrose and levulose actively, raffinose moderately and inuline with difficulty. It has no action on maltose or lactose. It inverts saccharose rapidly and ferments it. In wort at 25° C. *D. globosus* produces a rather rapid fermentation. After five days, it forms 1.25 per cent of alcohol by volume, and after 8 days 1.30 per cent.

DEBAROMYCES TYROCOLA. Konokotin

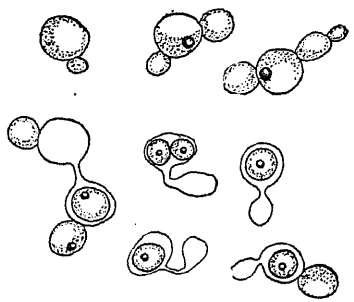


Fig. 91–A. — *Debaromyces tyrocola* (after Konokotin).

This yeast was isolated from Dutch cheese prepared in Russia. It is a yeast in which the copulation tends to become heterogamic. The copulation may be either iso- or heterogamic but the latter seems to be most frequent. It is accomplished as in the preceding species. The ascs usually contain a single ascospore which germinates by budding. This species ferments dextrose, levulose, galactose, saccharose and lactose.

Genus IV. Nadsonia

Ascs preceded by a heterogamic copulation between a mother cell (macrogamete) and a bud from it (microgamete). The macrogamete forms a bud which changes into an asc provided with one ascospore having a verrucose wall.

NADSONIA FULVESCENS. Sydon

Syn. GUILLIERMONDIA FULVESCENS (Nadson and Konokotin) [1]

This yeast was found along with *Endomyces magnusii*. It bears some resemblance to *Debaromyces globosus* but differs from it in its sporulation and Nadson and Konokotin have created a new genus for it.

It is a yeast with oval cells, elliptical or shaped like a lemon.

[1] Nadson, G. A. and Konokotin, A. G. Guilliermondia, eine neue Hefengattung mit heterogamer Kopulation. Cent. Bakt. 34 (1912) 241–242.

The asc is derived from a heterogamic copulation of two cells. The female cell or macrogamete is an adult cell and the male cell or microgamete is a small bud formed by the macrogamete. Both cells or gametes become united by means of a copulation canal. The contents of the microgamete enter the macrogamete from which an egg results. This then changes into an asc. The asc contains a single ascospore. The spore is spherical with a large globule of fat in its center. Its membrane is rough with little elevations and has a reddish brown color. On account of this color, it is easy to recognize macroscopically a culture which has sporulated. During germination the spore swells up and breaks open the wall. Liberated, it develops a germinating tube and produces a vegetative cell. This yeast has both sporogenic and asporogenic types. The asporogenic type develops into colonies (giant) which are distinguished by their white color from those of the sporogenic which are reddish brown. This species ferments dextrose, galactose, levulose and saccharose slowly.

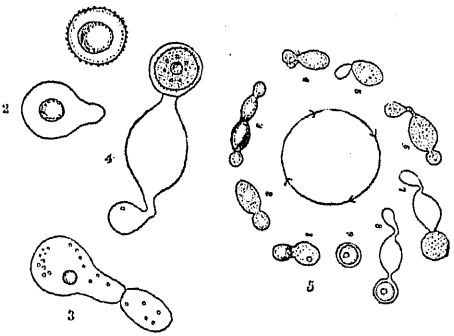


Fig. 91–B. — *Nadsonia fulvescens* (after Nadson and Konokotine).
1–3, Germination of Spores; 4, Ascs; 5, Cycle.

NADSONIA ELONGATA. Konokotin

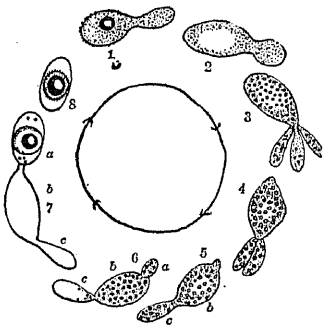


Fig. 91–C. — *Nadsonia elongata* (after Konokotine).

This yeast possesses vegetative cells which are oval or elongated. Copulation is accomplished as in *Nadsonia fulvescens*. The asc results from an egg containing a single spore. These ascospores have a very verrucose membrane and germinate by ordinary budding. The giant colonies on peptone gelatin with 5 per cent of glucose are rosette-shaped. They have brown centers and white peripheries. This species ferments dextrose and levulose, but has no action on other sugars.

Genus V. Schwanniomyces. Klöcker

Ascs derived from cells which have preserved a trace of sexual attraction. Ascospore provided with a single membrane on the sur-

face of which are small elevations, and also provided with a projecting collar. For germination, one of the halves of the ascospore swells and it is thus that budding is accomplished.

SCHWANNIOMYCES OCCIDENTALIS. Klöcker

This species was found by Klöcker [1] in the same environment as *Debaromyces globosus*. It has elliptical or spherical cells (5 to 10 μ), but some cells may appear rarely as elongated sausages. After a month's sojourn at room temperature on beer wort gelatin, the giant colonies appear well developed with a grayish white appearance. They resemble wax and have a glistening appearance.

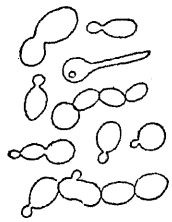


Fig. 92. — *Schw. occidentalis* (after Klöcker).

Sporulation is easily accomplished on plaster blocks. The limits of temperature for ascospore formation on plaster blocks are: minimum, 10 to 13° C.; maximum, 34° to 36° C. The ascs are always provided with a projection which gives them the appearance of a retort by means of which, during sporulation, they unite two by two. (Fig. 93.)

Guilliermond [2] has shown that these formations should be regarded as traces of an ancestral copulation. The cells destined to form ascs retain a little of their sexual attraction and attempt to fuse. Finally, on account of insufficient sexual attraction, the cells are not able to establish an anastomosis and develop parthenogenetically. The ascs form usually a single ascospore rarely two. The ascospore has the shape of a slightly flattened bowl. It is surrounded by a projecting collar which divides it into two unequal parts. The surface is rendered rugose by many little elevations. In the center is a globule of fat.

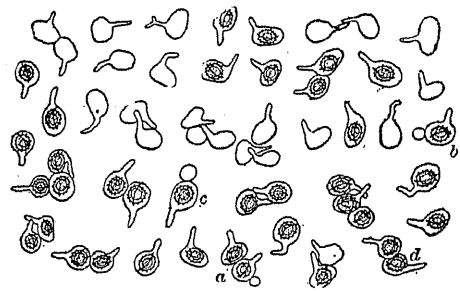


Fig. 93. — Formation of the Asc in *Schw. occidentalis*.

Germination commences by a swelling of the ascospore which localizes itself to the smallest half; this loses its elevations. Finally all of the elevations disappear (Fig. 41).

[1] Klöcker, A. Deux nouvelles genres de la famille des Saccharomyces. Comp. Rend. des trav. du lab. de Carlsberg, 8, 1909.

[2] Guilliermond, A. Sur un curieux exemple de parthenogenese observe dans une levure. Comp. Rend. Soc. Biol. 69, 1910; Quelques remarques sur la sexualite des levures. Annales mycologici, 8, 1910.

In old cultures on wort, this yeast forms a viscous scum more or less developed. Often the formation of a very thin ring may be noticed.

After a month's sojourn at room temperature, giant colonies on wort gelatin appear well developed with a grayish color. They resemble wax and possess a glistening appearance. The edge is slightly notched.

This species ferments dextrose, levulose, and raffinose, sometimes inuline, but has no action on lactose or maltose. It inverts sucrose more or less actively.

Genus VI. Torulaspora. Lindner

Cells round, spherical, small, provided with a large globule of fat and resembling *Torula*. These characteristics, as remarked by Klöcker; are insufficient to characterize the genus. However the trace of copulation which is present in the *Torulaspora*, recently pointed out by L. Rose, added to the characters described by Lindner, seem sufficient to differentiate this genus.

TORULASPORA DELBRÜCKII. Lindner

This species was discovered by Lindner[1] in English beer. (Fig. 94.) The ascospores are to the number of 3 to 5 in each asc. According to Rose, the ascs possess spurs analogous to those which have been observed in the *Schwanniomyces* which may be regarded as traces of copulation. This yeast is able to ferment dextrose, levulose, d-mannose and d-galactose.

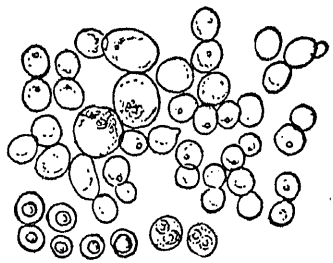


Fig. 94. — *Torulaspora Delbrückii* (after Lindner).

YEASTS E AND F. Rose

This species was isolated from the mucous secretions of oak trees by Rose[2] in 1910. Both present the characteristics of the genus *Torulaspora* and seem to be identical. They possess round cells (3.5 to 4.5 μ in diameter) and grow on beer wort quite well, but do not produce fermentation.

The ascs develop at the end of three days on Gorodkowa's gelatin and plaster blocks at 25° C. They show attempts at copula-

[1] Lindner, P. Mikroskopische Betriebskontrolle in den Gärungswerben. Paul Parey, edit. Berlin, 6th edition, 1909.

[2] Rose, L. Beitrage zur Kenntniss der Organismen in Eichenschleimfluss. Inaugural dissertation, University of Berlin, June 25, 1910.

tion and, like the *Schwanniomyces*, are supplied with a projection (Fig. 30). Often one may see a union of two of these projections from closely situated cells but no true union takes place on account of the persistence of a wall, and each forms a parthenogenetic asc.

The giant colonies are flat with small verrucose elevations. This yeast ferments dextrose, levulose, d-mannose, and saccharose and sometimes raffinose, trehalose, and inuline.

Rose has observed traces of copulation quite similar in a yeast isolated by Lindner from the secretions of a tree in the Berlin botanical garden and described in his atlas as *Torula sporogene* related to *Torulaspora Delbrückii*.

SACCHAROMYCES LACTIS γ. Dombrowski

This yeast isolated by Collau from sour cream butter has been described by Dombrowski.[1] It possesses no characteristics of the genus *Torulaspora*. However, as the ascs result from cells which have preserved a trace of copulation or sexual attraction, it should probably be classed along with the genera *Schwanniomyces* and *Torulaspora*, preserving the provisional name of *Saccharomyces lactis* (gamma). Perhaps it will be possible to create a new genus for it when it is better known. The cells are oval, sometimes spherical, and, on beer wort, are 5 to 6.5 μ in length and 5 μ in breadth.

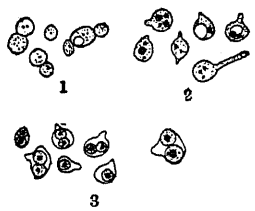


Fig. 95. — *Sacch. lactis* γ. 1, Vegetative Cells; 2, Cells destined to Form Ascs; 3, Ascs (after Dombrowski).

Sporulation is easily accomplished on plaster blocks, in old cultures on gelatin, and in the moist chamber. They appear in from 72 to 96 hours on plaster blocks at 25° C. The cells destined to sporulate show the presence of a projection more or less elongated which seems to represent a trace of ancestral copulation (Fig. 95, 2). They contain large fat globules. The ascs contain one or two ascospores, rarely three. (Fig. 95, 3.) The ascospores are shiny and contain a drop of fat in the center. Germination is accomplished by budding, during which the fat disappears.

In cultures on nutrient gelatin in plates, the colonies are round or shaped like a torpedo in which the edge enlarges in old cultures. In gelatin stabs the development is along the line of inoculation and becomes less and less as it progresses into the tube away from the surface. Giant colonies have a raised center and a border composed of concentric rings with slender rays.

[1] Dombrowski, W. Die Hefen in Milch und Milchprodukten. Cent. Bakt. 28, 1910.

In wort at the temperature of the laboratory, *Saccharomyces lactis* γ forms a ring and scum in which the cells possess the same characteristics as those in the sediment. This yeast acts like a top yeast. Fermentation is active at first but ceases quite rapidly. The wort is strongly discolored. No aroma seems to be formed. At the end of five months and a half, 5.4 per cent of alcohol is formed. In milk, this yeast produces no fermentation but provokes a strong peptonization of the casein. It ferments saccharose, dextrose, d-galactose, but has no action on maltose or lactose.

THIRD GROUP

Yeasts multiplying by budding, in which the ascs always form by parthenogenesis, all traces of sexuality having disappeared. Sometimes there is the formation of a rudimentary mycelium. In sugar solutions a deposit is formed and, very much more slowly, a scum, in which the vegetation is shiny without occluded bubbles of air. The ascospores are smooth, round or oval, with one or two membranes, germinating by budding or under exceptional conditions by a process intermediate between budding and partition. Germination of the ascospores is sometimes preceded by a fusion of these latter two by two (parthenogamy). The greater number of the species in this group produce the alcoholic fermentation.

Genus VII. Saccharomycodes. Hansen

Cells dividing by a process intermediary between budding [1] and partition. Often there are rudimentary myceliums with very distinct transverse walls. Ascopores fuse (parthenogamy) two by two at the moment of germination and develop in a single direction by a process intermediary between budding and partition.

SACCHAROMYCODES LUDWIGII. Hansen

Syn. SACCHAROMYCES LUDWIGII. Hansen

This species was discovered by Ludwig [2] in the mucous secretions of the oak in which he found associated a *Leuconostoc* and *Endomyces Magnusii*. Ludwig first regarded it as a form of *Endomyces mag-*

[1] By its manner of multiplication, the genus *Saccharomycodes* is then intermediate between the *Schizosaccharomyces* and the budding yeasts. It may also be possible to separate other yeasts into a separate group.

[2] Ludwig, F. Ueber der Alkoholgärung und Schleimfluss lebender Bäume. Bericht. der Deutsch. Bot. Gesells. 4, 1886.

nusii. Hansen [1] later isolated *Endomyces magnusii*. Rose has found this yeast since then under the same condition.

Saccharomyces Ludwigii possesses variable shape and dimensions; some cells are elliptical, others are elongated, tubular or with the shape of a lemon. (Fig. 96.) The cells multiply by a process intermediate between budding and transverse partition. They form generally at both extremities, rarely laterally, a projection, a sort of a bud which, when it has attained a certain size, separates itself from the mother cell by a thin wall accompanied by a slight tightening of the neck. The temperature limits for budding in beer wort are: minimum, 1–3° C.; maximum, 37–38° C.

In old cultures especially on gelatin, *Saccharomyces Ludwigii* shows a manifest tendency to produce well developed rudimentary myceliums which resemble a true mycelium. These formations are made up of a series of budding ramifying filaments. The cross walls are very marked but almost always accompanied by a slight constriction and the units easily separate. Each of the cells in the mycelium is able to bud and form ascospores. Long branching units with walls may be seen in the mycelium. (Fig. 5.) By the presence of these mycelial formations, *Sacch. Ludwigii* seems to offer an intermediate step between the *Endomyces* and the yeasts.

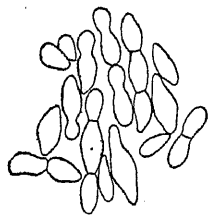


Fig. 96. — *Saccharomycodes Ludwigii* (after Lindner).

Ascospores form easily in water solutions of sugar, on wort gelatin in yeast water, on slices of carrot and even in liquid wort. They develop equally in numbers on plaster blocks.

According to Nielsen [2] the maximum temperature for sporulation on plaster blocks is 32° to 32.5°; the minimum is between 3° and 6° and the optimum between 30° and 31° C.

The ascs may contain from two to four ascospores, rarely more, but almost always there are four. These are round and about 3 or 4 μ in diameter. Germination is accomplished in a special manner which has been described at the beginning of the book and which will not be repeated here. It is generally preceded by a sexual process which Guilliermond [3] has described and which is comparable to parthenogamy. (Figs. 25 and 26.) After the ascospores have swelled they

[1] Hansen, E. C. Ueber die im Schleimflüsse lebender Bäume beobachteten Mikroorganismen. Cent. Bakt. 5, 1889.

[2] Nielsen, J. C. Sur le dev. des spores des S. membranaefaciens, anomolous et Ludwigii. Comp. Rend. des trav. du lab. de Carlsberg, 3, 1891.

[3] Guilliermond, A. Recherches sur la germination des spores et sur la conjugaison dans les levures. Rev. gen. de Bot. 17, 1905.

unite two by two. This fusion almost always operates between two ascospores from the same asc, exceptionally between two spores from closely situated ases. A canal for copulation is formed through which the contents fuse. The fusion takes place, the copulation canal gives birth to a sort of germinating tube which enlarges and takes the shape of a vegetative cell; it finally cuts itself off from the canal by a wall accompanied by a circular construction. (Fig. 36.) The cell thus formed separates from the zygospore which continues to form new cells by the same process. The fusion of the ascospores is generally not absolute and quite a number among them germinate alone.

We have observed, as has been stated, a species of *Saccharomyces Ludwigii* from Hansen's laboratory which, having remained for a time at laboratory temperature, had completely lost its sexuality. The ascospores always developed without fusing.

Hansen has shown that when various cells are cultivated from a colony of this yeast, a sporogenic and an asporogenic race may be obtained.

On wort gelatin, *Saccharomyces Ludwigii* develops in the shape of vegetative spots in which the color varies from a clear gray to a pale yellow. In beer wort, it produces at the end of about a month, at room temperature, a scum with elongated colonies.

It yields even after a fermentation of long duration, only 1.2 per cent of alcohol by volume. It does not act on maltose. On the other hand in glucose about 10 per cent of alcohol is produced. It inverts saccharose, and ferments dextrose, d-galactose, d-mannose, levulose, raffinose, and sometimes very slightly, l-sorbose, and tagatose (Lindner). It has no action on lactose or maltose.

SACCHAROMYCES BEHRENSIANUS. (Behrens)

This yeast, discovered by Behrens [1] on hops, possesses round or oval cells which divide like those of *Saccharomyces Ludwigii*. The optimum temperature for sporulation is from 18 to 20° C.; at this temperature, the ascospores appear in about 22 hours. The ascospores are spherical (4 to 4.5 μ in diameter) and are to the number of two or three in an asc. Their germination is accomplished as in *Saccharomyces Ludwigii*. This yeast produces no scum. On 10 per cent wort gelatin, the giant colonies present quite a characteristic appearance. They show fine concentric rings placed around a crateriform cavity which makes up the center. The edge of the colonies is of a pure white and the middle portions of a yellow color. In

[1] Behrens, J. Studien über die Konswevierung und Zusammensetzung des Hopfens. Woch. f. Brau. 13, 1896.

giant colonies, numerous ascospores are noticed. This yeast ferments dextrose, levulose, and maltose but does not act on saccharose, lactose, or d-galactose.

SACCHAROMYCES COMESII. Cavara

This species was described in 1893 by Cavara.[1] It grows parasitically and saprophytically in the panicles and stalks of millet. Cavara described ascs enclosing a varied number of ascospores. These ascospores fuse into one at the moment of their germination. Germination is accomplished as with *Saccharomyces Ludwigii* by the formation of a germinating tube. Guilliermond[2] has shown from the illustrations presented by Cavara, that this species is probably not yeast but probably a *Dematium*. Cavara regarded it as a yeast on account of the incorrect interpretation of forms in its development. *Saccharomyces comesii* is, then, not a yeast and should not be included in the family of *Saccharomycetes*.

Genus VIII. Saccharomycopsis (Schiönning)

Ascospores in two membranes germinating by budding.

SACCHAROMYCOPSIS GUTTULATUS (Robin) Schiönning

Syn. SACCHAROMYCES GUTTULATUS. Winter. CRYPTOCOCCUS GUTTULATUS. Robin

This species was discovered by Remarck and Robin and studied later by Buscalioni,[3] Casagrandi and Wilhelmi.[4] It seems to live as a true parasite in the intestinal canal of certain animals (birds, reptiles and mammals). It swarms in the intestinal canal of the rabbit, less frequent in guinea pigs, and appears in the excrement of these animals. *Saccharomyces guttulatus* possesses large cells, oval or more or less rectangular, resembling the oidia of *Oidium lactis*. The cells contain a large amount of glycogen and are often united in groups at their ends. (Fig. 97.) Budding

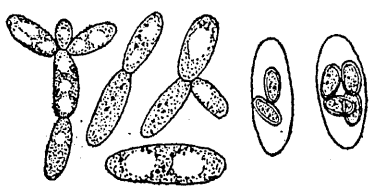


Fig. 97. — *Saccharomycopsis guttulatus* (after Buscalioni).

[1] Cavara. Sur un microorganisme zymogène de la Dourra. Revue mycologique, 1893.

[2] Guilliermond, A. Observations sur la germination des spores du *S. Ludwigii*. Bull. de la Société de Mycologie, 19, 1903.

[3] Buscalioni, L. Saccharomyces guttulatus. Giornale Malphigia, 10, 1896.

[4] Wilhelmi, A. Beitrage zur Kenntniss des Saccharomyces guttulatus. (Buscalioni) Inaugural dissertation. Bern. Cent. Bakt. 4, 1898.

takes place at both ends of the cells. The optimum temperature for budding is from 35° to 37° C. A scum formation has not been observed. Sporulation has been observed in the excrements of rabbits. Ascospores are formed to the number of one to four in each asc. They are oval, elongated and, according to Wilhelmi, are surrounded with a double membrane, an exosporium and an endosporium. Wilhelmi has been able to cultivate *Saccharomyces guttulatus* in various artificial media. It grew especially well in glycerol gelatin to which tartaric acid and dextrose had been added. It inverts saccharose and ferments dextrose. Casagrandi and Buscalioni have noted its pathogenic properties on subcutaneous injections into guinea pigs, rats and rabbits.

Genus IX. Saccharomyces. Meyen

Ascospores in a single membrane germinating by budding. Sometimes a mycelium is produced with transverse walls.

A. First Sub-group

Yeasts fermenting saccharose, dextrose and maltose but having no action on lactose.

SACCHAROMYCES CEREVISIAE. Hansen [1]

Syn. S. CEREVISIAE I, Hansen. — S. CEREVISIAE, Meyen. — TORULA CEREVISIAE, Turpin. — CRYPTOCOCCUS FERMENTUM, Kutzing. — HORMISCUM CEREVISIAE, Bail. — S. CEREVISIAE, Rees

This species is a top yeast which was found by Hansen in breweries of London and Edinburgh and has been used for a long time in the making of beer. Hansen gave it the name *S. cerevisiae* because it resembled the yeast described under the same name by Rees and Meyen. Young cells in the sediment in beer wort are large and either round or oval. Elongated cells are not observed under these conditions. (Fig. 2.) The temperature limits for budding in beer wort are: minimum, 1–3° C.; maximum, 40° C.

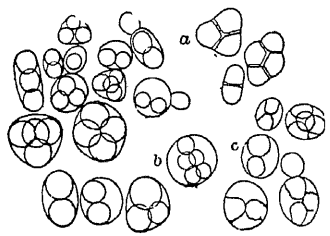


Fig. 98. — Asci of *S. cerevisiae* (after Hansen).

[1] Hansen, E. C. Recherches sur la morphologie et la physiologie des alcooliques ferments. II, Les ascospores chez le genre Saccharomyces. III, Sur la Torula de M. Pasteur. IV, Maladies provoques dans la bière par les ferments alcooliques. Comp. rend. du lab. de Carlsberg, Vol. 1, Book 2, 1883; Vol. 2, Book 4; Vol. 5, Book 2, 1909.

Temperatures for the formation of ascospores



Fig. 98–A. — Chondriome in *Saccharomyces cerevisiae.*

Fixation in a mixture of Potassium Dichromate and Formalin. Stained with Ferric haematoxyline.

37.5° C. no ascospores are formed

36–37	first	appearances	in	about	29 hours
35	"	"	"	25	"
33.5	"	"	"	23	"
30	"	"	"	20	"
25	"	"	"	23	"
23	"	"	"	27	"
17.5	"	"	"	50	"
16.5	"	"	"	65	"
11–12	"	"	"	10	days
9	no development				

The asci enclose from one to four ascospores, sometimes five. The ascospores are very refractive and possess a very distinct wall. (Fig. 98.) Their size varies from 2.5 to 6 μ.

Temperatures of Scum Formation

At 38° C. there is no formation of scum.

33–34	at	the	end	of	9–18	days,	very	slightly	developed
26–28	"	"	"	"	7–11	"	"	"	"
20–22	"	"	"	"	7–10	"	"	"	"
13–15	"	"	"	"	15–30	"	"	"	"
6–7	"	"	"	"	2–3	months	"	"	"

5 no formation of scum.

The cells in the scum have the following microscopic characteristics. At 20–34° C. the cells in the scum are elongated and possess an odd appearance. At 15 to 16° C., most of the cells look like those which were present when the inoculation was made. Some of the cells possess irregular shapes. In old scums all sorts of cells are visible. Some are extremely long, having the appearance of a mycelium. (Fig. 99.)

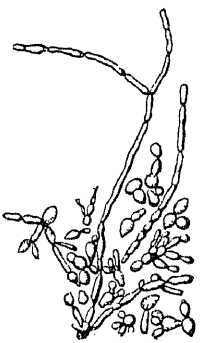


Fig. 99. — *S. cerevisiae.* Mycelial Formation in an Old Scum (after Hansen).

Yeasts like S. Cerevisiae

Numerous races and species of yeasts are known under the name of *S. cerevisiae.* Their systematic position is slightly unknown; a few of them will be mentioned here.

WILL'S YEASTS

Variety 2, *H. Will.*[1] Large round or oval cells. On gelatin the colonies are spherical or lenticular. Ascospores develop easily and abundantly. The temperature limits for the formation of ascospores on plates are 31° and 11° C., the optimum being near 25° or 26° C. The temperature limits for scum formation on beer wort are from 28–31° C. to 8–11° C. This variety is a bottom yeast which is an active fermenter.

Variety 6, *H. Will.* The cells of this variety are oval and sometimes curled. The colonies are spherical or lenticular on gelatin. Ascospores are formed easily and abundantly. The temperature limits for ascospore formation on plaster blocks are 31° and 11° C. The optimum is 28° C. The temperature limits for scum formation are 25–31° and 7–10°. This species is a moderate fermenter and a bottom yeast.

Variety 7, *H. Will.* The cells are round with giant cells mixed in. At the end of fermentation, chains may be seen composed of small cells in the budding stage. The colonies on gelatin are at first irregular with a winding embattled edge. Ascospores are formed with difficulty. The temperature limits for the formation of ascospores on plaster blocks are 30° and 13°. The optimum is around 25 or 26° C. The limits of temperature for the formation of scum on beer wort are 20–28° and 4–7° C. This variety is a bottom yeast with feeble fermenting ability.

Variety 93, *H. Will.* The cells are round or oval and the gelatin colonies spherical or lenticular. The temperature limits for the formation of ascospores on plaster blocks are 30° and 10° C. The optimum is 28° C. The temperature limits for scum formation on beer wort are 30–31° and 4–7° C. The scum contains numerous durable cells. This variety is a very active bottom yeast.

YEASTS OF SAAZ AND FROHBERG

Although insufficiently known from the standpoint of morphology, we should not pass these yeasts in silence for they play an important rôle in the industrial alcoholic fermentations. One was found in a Bohemian brewery (Saaz), the other in a brewery at Frohberg having been isolated by Lindner.[2] Since that time they have been

[1] Will, H. Vergleichende Untersuchungen an vier untergärigen Arten von Bierhefe. Zeitschr. f. d. ges. Brauw. 18, 1896.

[2] Lindner, P. Mikroskopische Betriebskontrolle in den Gärungswerben. Paul Parey, Berlin, 6th Edition, 1909.

studied by various investigators as Delbrück, Irmisch, Lindner, Reinicke, etc. The Saaz yeast is a bottom yeast by attenuation and ferments dextrose, d-mannose, d-galactose, levulose, maltose, saccharose, trehalose, melibiose, raffinose and α-methylglucoside.

A large number of industrial species (brewery and distillery) related to these species have been described. Some produce a top fermentation while others produce a bottom fermentation. Bau has made four groups: 1, Bottom yeasts of the Saaz type; 2, Top yeasts of the Saaz type; 3, Bottom yeasts of the Frohberg type; 4, Top yeasts of the Frohberg type. The yeasts of the first two groups are characterized by the fact that they give a feeble attenuation, those of the second two groups that they give a strong attenuation.

Different industrial yeasts have been described by Van Laer, Jörgensen, Greg, and a few other authors. Among the best known may be mentioned variety II and XII, top distillery yeasts which have been isolated at the Berlin Institute of Fermentology.

SACCHAROMYCES CARLSBERGENSIS. Hansen

Syn. CARLSBERG YEAST. Hansen

Described for a long time by Hansen[1] under the provisional name of Carlsberg Yeast I, this species has been subjected to a careful study by the same author who has given it the name of *Saccharomyces Carlsbergensis*. This yeast like the *Saccharomyces monacensis* which we shall describe shortly, has been used for a long time in Copenhagen breweries. After culturing for 24 hours on beer wort at 25° C., or after two days at the temperature of the laboratory, *Saccharomyces carlsbergensis* forms a doughy sediment made up of elliptical cells, shaped like an egg or pear. (Fig. 102.) Cells with small points make up the characteristic shape of this variety. The temperature limits for budding on beer wort are 33.5° C. and 0° C.



Fig. 100. — *S. carlsbergensis*. A Wort Culture of 24 Hours at 25° (after Hansen).

In the neighborhood of the minimum temperature, this yeast forms mycelial cells in chains with elliptical cells intermixed. Giant cells are rare. The mycelial cells form between 0° and 9° C. (Fig. 101.) They appear after 2 or 3 months at 0.5° C. At 1 or 2° C. they are formed in a month and a half and a little more quickly at 3 or 4° C.

[1] Hansen, E. C. Recherches sur la physiologie et morphologie des ferments alcooliques, XIII. Nouvelles études sur des levures de brasserie à fermentation basse. Comp. Rend., lab. de Carlsberg, 7, 1908.

At the maximum temperature, the cells have the same shape as the cells used in the inoculation except that they are a little larger. On the other hand giant cells are more numerous. (Fig. 102.) Ascospores are formed but rarely and in small numbers. It is not possible to determine the temperature limits for their development. A feeble formation of scum is obtained in Pasteur flasks after a month at 13° to 15° and at laboratory temperature. At the end of two months, little islands of scum have formed made up of spherical and elliptical cells. At the end of one or two years, the old scum covering cultures maintained at the temperature of the laboratory shows the same characteristics. The cells are always spherical or ellipsoidal and the chain formation is very rare.

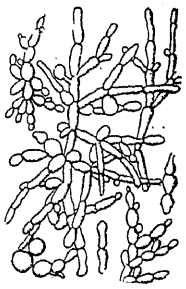


Fig. 101. — *S. carlsbergensis*. Vegetation after Two or Three Months in Beer Wort at 0.5° C. (after Hansen).

Giant colonies on wort gelatin offer the form of a rosette. More often they present in the center a depression which is surrounded by a number of concentric rings. The edge of the colony is slightly undulated. Some of the colonies have a glossy surface while others have a scaly appearance. They are ordinarily dry and of a chalky appearance. Liquefaction of gelatin is not produced in three months.

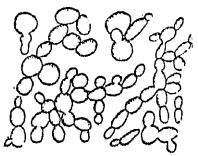


Fig. 102. — *S. carlsbergensis*. Vegetation of a Culture after two to three months in Beer Wort at 32–35° C. (according to Hansen).

Cultures on plates or on gelatin produce colonies of the shape and size of pinheads. The superficial colonies have a grayish yellow color and a chalky appearance. This yeast ferments dextrose, saccharose, maltose and galactose, but not lactose; it is a bottom yeast and an active fermenter.

SACCHAROMYCES MONACENSIS. Hansen [1]

This species described for a long time under the provisional name of Carlsberg Yeast II has recently been restudied by Hansen and given the definite name of *Saccharomyces monacensis*. In cultures on beer wort, it forms at the end of 24 hours at 25° C. a thin waxy sediment made up mostly of ellipsoidal or round cells. Giant cells are rather frequent.

The temperature limits for budding in beer wort are 33° and 1° C.

[1] Hansen, E. C. Recherches sur la morphologie et la physiologie des ferments alcooliques. XIII. Nouvelles etudes sur des levures de brasserie a fermentation basse. Comp. Rend. du lab. de Carlsberg, 7, 1908.

At the maximum temperature, this yeast has ellipsoidal cells predominating with a few in the form of chains. The cells are larger and longer (Fig. 103, 1); at the minimum temperature, spherical and ellipsoidal cells may be seen at the end of 18 days. At the end of a month, one may find a small number of colonies composed of cells in a short chain with a few rare giant cells. (Fig. 103, 3.)

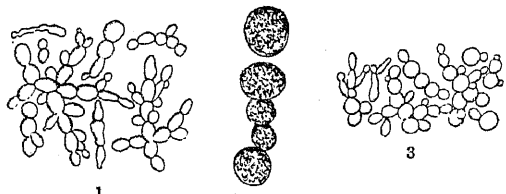


Fig. 103. — *S. monacensis.*

1. Vegetation of a Culture on Beer Wort after 2 to 3 Days at 32–33°C. — 2. Giant Cells Obtained in a Culture of 10 Days in Yeast Water to Which Dextrose Was Added at 9° C. — 3. Vegetation after 16 Days on Beer Wort at 1–2° C. (after Hansen).

In yeast water to which dextrose has been added, one may notice after about 12 days at 9° C. a feeble formation of a mycelium. On the other hand, in this medium giant cells are so numerous and so large that they serve as a distinguishing characteristic between this species and the preceding one. (Fig. 103, 2.)

At 13° to 15° C., one may see at the end of about a month in a Pasteur flask, the beginning of a scum with the form of floating islands in which the cells are spherical or ellipsoidal. At the end of a year, one may see in cultures, maintained at laboratory temperatures, an abundant scum formation which generally covers about all of the surface. These scums are again composed of cells which are ellipsoidal or round, chain formation being extremely rare.

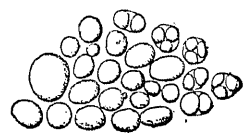


Fig. 104. — *S. monacensis*, with Asci (after Hansen).

Ascospore formation is accomplished more easily in this species than in the preceding one, without being abundant. (Fig. 104.) But it is impossible to determine the temperature limits of this formation.

Giant colonies on wort gelatin have much the appearance of those of the preceding species. They take the shape of rosettes with an undulating border. The colonies have a depressed center which is surrounded by a more elevated ring than with *S. carlsbergensis*. However the center of the colony consists more often of a wart and the points of the rosette are a little more pronounced than in *S. carlsbergensis*. *S. monacensis* is a yeast producing a typical bottom fermentation, fermenting dextrose, saccharose, maltose and not lactose.

SACCHAROMYCES PASTORIANUS. Hansen [1]

This is a species which is often encountered in the air in the vicinity of breweries. It contributes a bitter, disagreeable taste and bad odor to the beer. Thus it contributes to the diseases of beer and impedes the clarification. It resembles very much *Saccharomyces pastorianus* described by Rees and Pasteur but is not capable of being

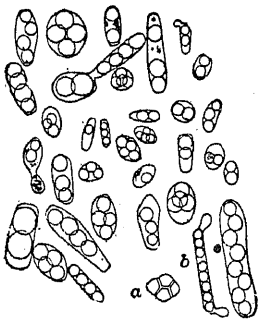


Fig. 105. — Ascs of *S. Pastorianus* (after Hansen).

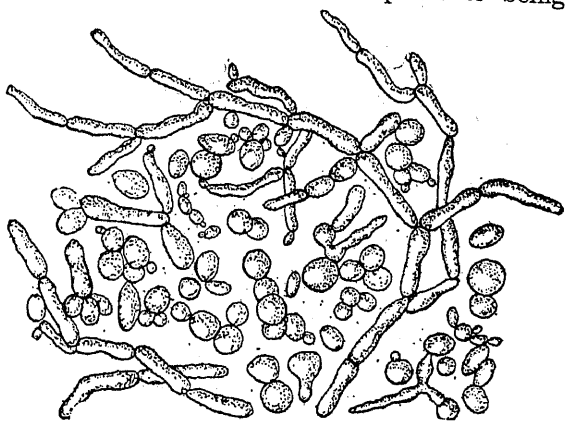


Fig. 106. *S. Pastorianus.* Vegetative Cells from Scum at 13–15°C. (after Hansen).

closely identified with this species. It forms in beer wort a sediment in which the cells are more or less elongated, mixed with large and small round or oval cells. (Fig. 3.)

The temperature limits for budding in beer wort are: minimum 0.5° C., maximum 34° C.

Temperatures for Ascospore Formation

At 31.5° C. no formation of ascospores.

29.5–30.5° first appearance of rudimentary ascospores in 30 hours.

29°	"	"	"	"	"	"	27	"
27.5°	"	"	"	"	"	"	24	"
23.5°	"	"	"	"	"	"	26	"
18°	"	"	"	"	"	"	35	"
15°	"	"	"	"	"	"	50	"
10°	"	"	"	"	"	"	89	"
8.5°	"	"	"	"	"	"	5	days
7.0°	"	"	"	"	"	"	6	"
3 –4°	"	"	"	"	"	"	14	"

0.5° no formation of ascopores.

[1] Hansen, E. C. Recherches sur la physiologie et la morphologie des ferments alcooliques. II. Les ascospores chez le genre Saccharomyces. III. Sur la Torula de M. Pasteur. IV. Maladies provoques dans la bière par les ferments alcooliques. Comp. Rend. du lab. de Carlsberg. Vol. 1, Book 2, 1883; Vol. 2, Book 4, 1886; Cent. Bakt. Vol. 5, Book 2, 1909.

The ascs are elongated (Fig. 105) and possess a number of ascospores which vary from 1 to 4 and may attain the number of 5 or 10. The size of the ascospore is quite variable; it varies between 1.5 and 3.5 microns and goes rarely to 5.0 microns. The ascospores never undergo copulation, which distinguishes *S. pastorianus* from *S. intermedius* and *S. validus* (*Marchaud*).

Temperatures of Scum Formation

At 34°	no scum formation.								
26–28°	at the end of	from	7	to	10	days,	feebly	developed.	
20–22°	" " " " "		8	to	15	"	"	"	
13–15°	" " " " "		15	to	30	"	"	"	
6–7°	" " " " "		1	to	2	months	"	"	
3–5°	" " " " "		5	to	6	"	"	"	
2–3°	no formation of a scum.								

The microscopic appearance of the cells in the scum is as follows: At 20–28° C. the cells present the same shape as in the deposit. At 13 to 15° C., vigorous colonies are seen having the appearance of a mycelium composed of ordinary cells elongated and in the form of chains (Fig. 106). In old cultures of scums, the cells are smaller than in the sediment. One finds queer cells sometimes almost filiform. This yeast ferments saccharose, dextrose, levulose and maltose.

SACCHAROMYCES INTERMEDIUS. Hansen [1]

Syn. SACCHAROMYCES PASTORIANUS II. Hansen. Also Rees

This yeast produces a feeble top fermentation. It was discovered by Hansen in the air of breweries in Copenhagen. It does not seem to cause any disease in the beer. Hansen has provisionally named it *Saccharomyces pastorianus* II. In wort it forms a sediment of elongated cells in chain formation. (Fig. 107.) One finds also large and small round or oval cells. The temperatures of budding on beer wort are: minimum, 0.5° C.; maximum 40° C.



Fig. 107. — *S. intermedius*. Young Cells from Sediment in Wort.

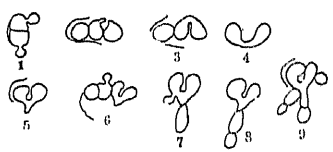


Fig. 107–A. — Germination of Ascospores in *Saccharomyces intermedius*.
1, Without Copulation; 2–9, After Copulation (according to Marchaud).

[1] Hansen, E. C. See references for Saccharomyces pastorianus.

Temperatures for the Formation of Ascospores

At 29° C. no formation of ascospores.

27–28°	appearance	of	first	rudiments	in	34	hours.
25°	"	"	"	"	"	25	"
23°	"	"	"	"	"	27	"
17°	"	"	"	"	"	36	"
15°	"	"	"	"	"	48	"
11.5°	"	"	"	"	"	77	"
7°	"	"	"	"	"	7	days
3–4°	"	"	"	"	"	17	"

0.5° no formation of ascospores.

The ascs are often elongated and include a variable number of ascospores (1 to 7) which measure 2 to 5 microns in diameter. (Fig. 108.) The ascospores undergo a copulation at the moment of germination in about half of the cases.

Temperatures for the Formation of Scum

At 34° C. no formation of ascospores

26–28°	at	the	end	of	7–10	days	feeble	development.
20–22°	"	"	"	"	8–15	"	"	"
13–15°	"	"	"	"	10–25	"	"	"
6–7°	"	"	"	"	1–2	months		
3–5°	"	"	"	"	5–6	"		

2–3° no formation of scum.

Microscopic appearance of cells in the scum is as follows: At 20–28° C. the cells of the scum present almost the same shape as those in the sediment. One may see queer shapes in the forms of chains. At 15 to 3° C. oval or round cells predominate. In the scums from old cultures, the cells are almost all smaller than those in the sediment. In yeast water gelatin, on streaks at 15° C., this yeast gives, at the end of about 16 days, a vegetation with relatively even edge which differs from *Saccharomyces validus*. It ferments dextrose, d-mannose, levulose, d-galactose, saccharose and maltose.

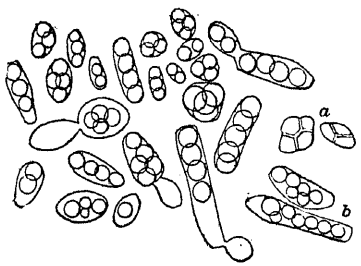


Fig. 108. — Ascs of *S. intermedius* (after Hansen).

SACCHAROMYCES VALIDUS. Hansen

Saccharomyces validus, at first called *S. pastorianus III* is a top yeast isolated by Hansen [1] from a bottom fermentation in beer where it caused cloudiness. In the vegetation at the bottom of beer wort the cells are ordinarily elongated in the form of sausages intermingled with a large or small number of round or oval cells. (Fig. 109.) The temperature limits of budding are: minimum, 0.5° C., maximum 39–40° C.

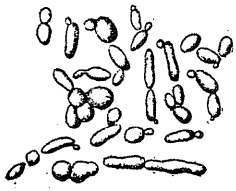


Fig. 109. — *S. validus*. Young Cells from Sediment in Wort (after Hansen).

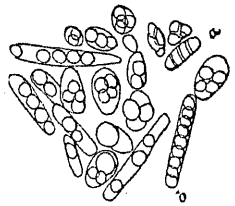


Fig. 110. — Asci of *S. validus* (after Hansen).

Temperatures for the Formation of Ascospores

At 29° C. no development of ascospores.

27–28°	appearance	of	first	rudiments	in	about	35	hours
26.5°	"	"	"	"	"	"	30	"
25°	"	"	"	"	"	"	28	"
22°	"	"	"	"	"	"	29	"
17°	"	"	"	"	"	"	44	"
16°	"	"	"	"	"	"	53	"
10.5°	"	"	"	"	"	"	7	days
8.5°	"	"	"	"	"	"	9	"

4° no development of ascospores.

The asci are ellipsoidal or elongated. The ascospores measure 2–4 μ in diameter and are variable in number in each asc (1 to 10) (Fig. 110.) Sometimes they germinate after having undergone a copulation (Marchand) but more often singly.

Temperatures for Formation of Scum

At 34° C. no formation of scum.

26–28°	at the	end of	7–10	days	feeble	development	
20–22°	"	"	"	9–12	"	"	"
13–15°	"	"	"	10–12	"	"	"
6–7°	"	"	"	1–2	months	"	"
3–5°	"	"	"	5–6	"	"	"

2–3° no formation of scum.

[1] Hansen, E. C. See references under S. pastorianus.

Microscopic appearance of the cells in the scum follows: At 20–28° C. the cells in the scum have almost the same shape as those in the sediment. At 15 to 3° C. and in old scums, one may see colonies composed of elongated cells in the shape of sausages in which the appearance is much like a mycelium. (Fig 111.) In yeast water gelatin, on streaks, one may see at the end of sixteen days a very irregular border. This yeast ferments d-mannose, levulose, d-galactose, saccharose and maltose.

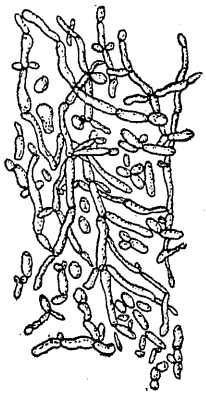


Fig. 111. — *S. validus.* Vegetative Cells from Scum at 13–3° C. (after Hansen).

LOGOS YEAST. Van Laer and Denamur

Here we shall again mention a species whose morphology is very little known but which is of very much importance industrially. It was isolated by Van Laer and Denamur[1] from among the yeasts used in the brewery of Logos and Company of Rio de Janeiro in Brazil. Its origin is unknown; it seems to have originated in a spontaneous fermentation of sugar-cane juice. It has the shape of *S. pastorianus.* During the fermentation the cells remain attached in large masses which settle to the bottom of the fermentation vats. It is a bottom yeast with a slow fermentation which produces little alcohol. It is very much attenuated. It ferments dextrine, inuline, dextrose, d-mannose, d-galactose, levulose, saccharose, maltose, raffinose, melibiose, and α-methylglucoside.

SACCHAROMYCES ELLIPSOIDEUS. Hansen

Syns: S. ELLIPSOIDEUS I. Hansen. — S. ELLIPSOIDEUS. Reess

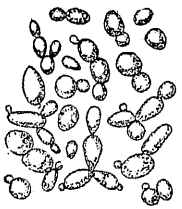


Fig. 112. — *S. ellipsoideus.* Young Cells from Sediment in Beer Wort (after Hansen).

This yeast was discovered by Hansen[2] on the surface of raisins. It is a bottom yeast which plays an important rôle in vinification. In the sediment in wort cultures, it possesses elliptical or round cells; elongated cells are not common. (Fig. 112.) The temperature limits for budding on beer wort are: minimum, 0.5° C., maximum, 40–41° C.

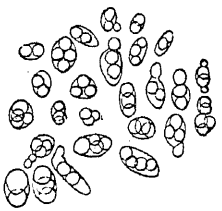


Fig. 113. — Asci of *S. ellipsoideus* (after Hansen).

[1] Van Laer, H., and Denamur. Notice sur une levure atténuation, limite très élevée. Moniteur scientific, 1895.

[2] Hansen, E. C. See references for *S. pastorianus.*

Temperatures for Ascospore Formation

At 32.5° no development of ascospores.

30.5–31.5° appearance of first rudiments in about 36 hours.
29.5° " " " " " " 23 "
25° " " " " " " 21 "
18° " " " " " " 33 "
15° " " " " " " 45 "
10.5° " " " " " " 41 days
7.5° " " " " " " 11 "
4° no development of ascospores.

The ascs are ordinarily ellipsoidal and small. They enclose from one to four ascospores which measure 2 to 5 μ (Fig. 113). They germinate after having copulated two by two (Marchaud) in about half of the cases.

Temperature for Formation of Scum

At 38° no formation of scum.
33–34° at the end of 8–12 days fully developed.
26–28° " " " " 9–16 " " "
20–22° " " " " 10–17 " " "
13–15° " " " " 15–30 " " "
6–7° " " " " 2–3 months
5° no formation of scum.

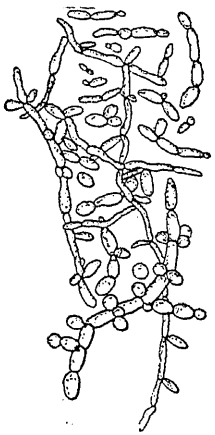


Fig. 114. — *S. ellipsoideus*. Vegetative Cells from Scum at 13–15°C. (after Hansen).

Microscopic appearance of the cells in the scum is as follows: At 20–34° C. and at 6–7° C., the scum includes cells which are small among which the sausage-shaped cells are abundant. At 13–15° C. in old scums, one may see branching colonies with sausage-shaped cells, either short or elongated. (Fig. 114.) In beer wort to which has been added 5½ per cent of gelatin, on streaks at 25° C., one may very closely separate the cells of the preceding species. (*S. cerevisiae, Pastorianus, intermedius* and *validus*) by a peculiar structure in the form of a network which is not able to escape the naked eye. This yeast ferments saccharose, dextrose and maltose.

YEASTS RELATED TO *S. Ellipsoideus*

Numerous yeasts are used in the making of wine which are related to *S. ellipsoideus*. They have been described by Aderhold, Hotter,

Lendner, Marx, Müller-Thurgan, Nastjukow, Osterwalder, Seifert, Wortmann, Jacquemin, Kayser and Jorgensen.

The most characteristic are the Johannisberg I and II yeasts, and *S. vini Muntzii.* We shall mention these rapidly.

JOHANNISBERG YEAST I. Wortmann [1]

This species possesses round, oval, or pointed cells. It forms a scum composed of oval cells at 26–27° C. The ascospores appear at 25–26° C. at the end of 28 to 30 hours. They germinate according to Marchaud after having copulated.

JOHANNISBERG YEAST II. Wortmann

This yeast has been described by Wortmann and Aderhold.[2] It possesses oval cells longer than the preceding yeast, but never pointed. The limits of temperature for budding on beer wort, according to Hansen, are 37–38° and 0.5° C. Ordinarily it is a bottom yeast. It sporulates very abundantly on the plaster block. The temperature limits for sporulation are 2.3° and 33–34.5°. The ascospores are to the number of four in each asc. It has been shown that they fuse more often two by two before germinating and undergoing a true copulation (parthenogamy) [3] (Fig. 35). Germination is accomplished by budding at some point on the copulation canal. This species forms a scum composed of round or sausage-shaped cells.

SACCHAROMYCES VINI MUNTZII. Kayser

This yeast was found by Kayser [4] on grapes. It is made up of cells in chains which possess a vacuolar protoplasm and die at about 55°. The ascospores form at the end of about 42 hours at 25°. This

[1] Wortmann, J. Landw. Jahrbucher, XXI, 1892.

[2] Aderhold, R. Die Morph. der deutschen Sacch. ellipsoideus Rassen. Landw. Jahrbucher, XXIII, 1894.

[3] In this yeast the fusion of ascospores exhibits very curious characteristics. In a certain number of cases the zygospore, formed by the union of two ascospores, commences to germinate before nuclear fusion has commenced. The two nuclei take a position in the middle of the copulation canal and fuse when the first bud forms. At the time when nuclear fusion takes place the nucleus which results from it quickly elongates similar to a bud and divides by amitosis in such a way as to furnish a nucleus to the bud. Sometimes, however, nuclear fusion does not seem to be accomplished. The two nuclei join and seem to divide simultaneously in a manner to form four nuclei, two of which remain in the zygospore and the other two enter the bud.

[4] Kayser, E. Contr. à l'étude des levures de vin. Ann. de l'Institut Pasteur, t. VI, 1892, et les Levures, Masson et Gauthier-Villars, éditeurs, 2e éd., 1905.

yeast ferments saccharose, dextrose, levulose and maltose. It possesses characteristics of a top yeast. The ascospores according to Marchaud undergo a copulation just before they germinate.

SACCHAROMYCES TURBIDANS. Hansen

Syn: S. ELLIPSOIDEUS II. Hansen. — S. ELLIPSOIDEUS. Reess



Fig. 115. — *S. Turbidans.* Young Cells from Sediment in Beer Wort (after Hansen).

Ordinarily this is a bottom yeast which was found by Hansen[1] in beer and first described under the name of *S. ellipsoideus I.* He found a very evident trouble in beer and this yeast may be regarded as an unfavorable species, more so than *S. validus.* In sediments in beer wort it always has round or elliptical cells. Elongated cells are rare. (Fig. 115.) The temperature limits for budding in beer wort are: minimum, 0.5° C., maximum 40° C.

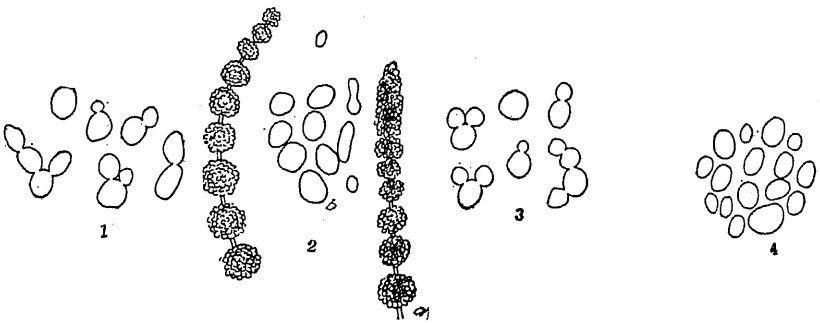


Fig. 115-A. — *Parasaccharomyces Ashfordii,* Anderson.

1, Cells from Young Beer Wort Culture. — 2, *a,* Moniliform Clusters beneath the Surface of an Old Agar Plant; *b,* Cells from Surface of the Same Culture. — 3, Young Cells. — 4, Old Cells.

Temperature Limits for Ascospore Formation

At 35° C. no ascospore formation.

33–34° appearances of first rudiments in 31 hours.
33° " " " " " 27 "
31.5° " " " " " 23 "
29° " " " " " 22 "
25° " " " " " 27 "
18° " " " " " 42 "
11° " " " " " 5½ days
8° " " " " " 9 "

4° no ascospore formation.

[1] Hansen, E. C. See references under S. pastorianus.

The ascospores are usually 5 μ in diameter (Fig. 116). According to Marchaud, they germinate after having copulated.

Temperatures for Formation of Scum

At 40° no formation of scum.
36–38° at the end of 8–12 hours feeble formation.
33–34° " " " " 3–4 " " "
26–28° " " " " 4–5 " " "
20–22° " " " " 4–6 " " "
13–15° " " " " 8–10 " " "
6–7° " " " " 1–2 months
3–5° " " " " 5–6 "
2–3° no formation of scum.

The microscopic appearance of the cells in the scum varies. At the beginning the cells look like those in the sediment but, as a rule, they are a little longer. In old scums, one may see colonies with short and long cells or tubular cells with branching projections. We have seen that Hansen was able to transform this yeast, normally a bottom yeast, into a top yeast.



Fig. 116. — Asci in *Saccharomyces turbidans* (after Hansen).

SACCHAROMYCES WILLIANUS. Saccardo [1]

Syn: SACCHAROMYCES I OF WILL. Bay [2]

The species has been described and named by Will,[3] with the provisional name of yeast No. 811. It is a bottom yeast related to *S. turbidans*. Its cells are egg-shaped.

The limits of temperature for the formation of ascospores on plaster blocks are 39–41° C. and 4–9° C. The optimum is 34° C. At this temperature sporulation appears at the end of 11 hours. The ascospores measure 1.5 to 5 μ in diameter, more often 3–5 μ. They appear at first very refractive, homogeneous, and show vacuoles with fat globules. Their number never exceeds four for each asc. They germinate ordinarily after having copulated. The temperature limits for the formation on beer wort of a scum are 39–41° C. and 4–5° C. The cells of the scum are elongated and form branching colonies. The

[1] Saccardo, P. A. Syllage fungorum. Padoue, II, 1895.

[2] Bay, J. C. The sporeforming species of the genus Saccharomyces. The American Naturalist, XXVII, 1893.

[3] Will, H. Zwei Hefearten welche abnorme Veränderungen in Bier veranlassen. Zeitschr. f. d. ges. Brauw., XIV, 1891.

colonies develop on wort gelatin with irregular shapes and with the appearance of a network with large meshes. Later the center becomes compact with irregular contours. The thermal death point for the vegetative cells is about 70° C. This species produces a disagreeable taste in beer and causes very pronounced difficulties.

SACCHAROMYCES BAYANUS. Saccardo

Syn: SACCHAROMYCES II OF WILL. Bay

This species was described by Will at the same time as the preceding one. He designated it provisionally as Yeast II. It also belongs to the type ellipsoideus. The cells have the shape of pointed eggs about 7 to 11 μ long and 5 to 6 μ wide. The temperature limits for the formation of ascospores on plaster blocks are 30–32° and 0.5–3° C. The optimum is 24–25° C. At this temperature, the ascospores appear at the end of 30 hours. They are to the number of 1 to 4 per asc and attain 2 to 4 microns in diameter. They germinate usually after having copulated. In old scums, the cells form budding colonies with branches. They are able to reach 30 microns in length and 2 to 3 microns in diameter. The thermal death point for vegetative cells is about 70° C. This species causes cloudiness in beer and at the same time produces a disagreeable aromatic odor, which is similar to that of decayed fruit, and an extremely astringent taste.

SACCHAROMYCES ILICIS. Grönland

This species was found by Grönland[1] on the fruits *Ilex aquifolium.* It is a bottom yeast with generally spherical cells. The temperature limits for sporulation are 8–9.5° and 36–38°. The ascospores are devoid of vacuoles. The scum contains cells which are slightly elongated. Streak cultures on gelatin have a farinaceous appearance. In beer wort this yeast produces about 2.8 per cent of alcohol by volume. Its vegetation gives a very disagreeable taste.

SACCHAROMYCES AQUIFOLII. Grönland

This yeast was also found by Grönland in the fruit *Ilex aquifolium.* It is a top yeast with large round cells. The temperature limits for the formation of ascospores are 8°–10.5° and from 27.5° to 31°. The ascospores possess vacuoles. The scum is made up of spherical and oval cells. Streak cultures on gelatin have variable appearances. This species in wort gives a very disagreeable taste. It produces in wort about 3.7 per cent of alcohol by volume.

[1] Grönland, C. En ny Torula-Art og to nye Saccharomyces-Arter Vidensk. Med. fra den Naturh. Foren, Copenhagen, 1892.

SACCHAROMYCES PIRIFORMIS. Marshall-Ward

Ward [1] isolated this yeast from ginger beer. It grows in symbiosis with *Bacterium vermiforme* and is found in the sheath of this organism. The bacterium seems to destroy certain substances which are detrimental to the yeast. This yeast possesses ellipsoidal or round cells like those of *Saccharomyces ellipsoideus*. The temperature limits for budding are 35° and 10° C. It sporulates on plaster blocks at the end of 24 hours at 25° C. Ordinarily the asci contain 4 ascospores. It causes an active fermentation in saccharose solutions and gives a white waxy sediment. In beer wort, it produces only a feeble fermentation and gives a scum made up of cells shaped like pears or small sausages.

SACCHAROMYCES VORDERMANNII.

Went and Prinsen-Geerligs

This yeast was discovered by Went and Geerligs [2] in a ferment used in Java for the manufacture of arrack. The cells are ellipsoidal in the form of an egg or onion. The asci enclose four ascospores. This species produces no scum in sugar solutions, but simply a ring. It yields about 10 per cent of alcohol. *Saccharomyces vordermannii* is the essential agent in the fermentation of arrak.

SACCHAROMYCES SAKÉ. Yabe [3]

Syn: SAKÉ YEAST. Kosai [4]

This yeast is used by the Japanese in the preparation of Saké from rice. The saccharification of the starch is accomplished by *Rhizopus oryzae*. The sugar thus obtained is finally decomposed to alcohol and CO_2 by means of the *Saccharomyces saké*. This yeast possesses spherical cells 6 to 12 μ in diameter. Ascospores, 6 to 12 microns in diameter, are secured on plaster blocks at 3–4° C. (minimum) and in 36 hours at 40°–41° C. (maximum), in 40 hours at 30–32° C. It easily ferments saccharose, maltose, levulose, dextrose, d-mannose, and α-methylglucosides and with difficulty trehalose and d-galactose. It decomposes raffinose into melibiose and levulose but does not hydrolize melibiose.

[1] Ward, M. The ginger beer plant and the organisms composing it. Philos. Trans. Royal Society, 183, 1898.

[2] Went, F., and Prinsen-Geerligs. Beobachtungen über die Hefearten und zuckerbildenen Pilze der Arrakfabrikation Verhand. d. Königl. Akad. d. Wetensch. te Amsterdam, 4, 1895.

[3] Yabe, K. Ueber den Ursprung von Sakehefe. Imperial University College of Agriculture Bulletin, 3, 1897.

[4] Kosai, Y. Chemie und biologie Unters., über Sakebereitung. Cent. Bakt. 1, 1900.

SACCHAROMYCES CARTILAGINOSUS. Lindner [1]

This species was found by Matthes in kephir grains. It gives a smoky taste to wort. Its cells possess a very granular protoplasm. The ascs contain 3 to 4 ascospores (Fig. 117). On beer wort, *S. cartilaginosus* forms at the end of a few weeks, on the surface of the liquid, small floating colonies of a firm consistency — almost cartilaginous. These unite and increase in size and result in making a scum. The yeast sediment is flocculent. The giant colonies are folded. This yeast ferments dextrose, d-mannose, levulose, saccharose and maltose but produces only feeble fermentation in d-galactose. It may also have an action on raffinose.

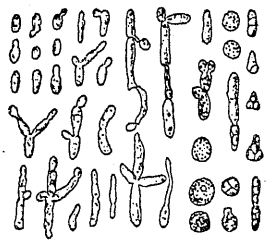


Fig. 117. — *Saccharomyces cartilaginosus*. Cells from Young Culture on Beer Wort, and Ascs (after Lindner).

SACCHAROMYCES BATATAE. Saito

This yeast was isolated by Saito [2] from moromi, a fermentable dough, made of a mixture of "koji" and potato (boiled) which is used in the manufacture of Brandevin (a wine in Japan). This yeast is the most active agent in this fermentation. The cells are oval and elliptical. In the scums they often have the shape of cells of *Saccharomyces pastorianus*. The ascospores form at the end of 24 hours at 25° C. They are spherical, very refractive and to the number of 2 or 3 in each asc. In beer wort at 25° C. this species produces 3 per cent of alcohol by volume. *Saccharomyces batatae* easily ferments dextrose, levulose, saccharose and maltose, more difficultly d-galactose, and raffinose; it has no action on melibiose, lactose, inuline and α-methylglucosides.

SACCHAROMYCES MULTISPORUS. Jörgensen. [3]

This is a wild yeast isolated by Holm from an English top yeast. Most of the cells are ellipsoidal. However, a large number are large round cells. These latter as well as the ellipsoidal, are capable of forming ascs. Spores appear on plaster blocks at the end of 40 hours

[1] Lindner, P. Mikroskopische Betriebskontrolle in den Gärungswerben, Paul Parey, Berlin, 6th edition, 1909.

[2] Saito, K. Mikro. Studien über die Zubereitung der Betatenbranntweins. Cent. Bakt. 18, 1907.

[3] Jörgensen, A. Die Mikroorganismen der Gärungsindustrie. 5th edition, Paul Parey, Berlin, 1909.

at 25° C. The asci formed by the ellipsoidal cells form only three or four ascospores; those from the large cells form nine or ten. The ascospores are round and very refractive. After long culturing in must sugar solutions, this yeast loses its ability to sporulate. *Saccharomyces multisporus* is a bottom yeast which adheres closely to the culture flask, so well that it is difficult to detach it. A thin scum is formed. It yields about 4 per cent of alcohol by volume and produces a disagreeable taste. This yeast ferments dextrose, maltose, and saccharose.

SACCHAROMYCES MALI RISLERI. Kayser [1]

Discovered in specimens of cider by Kayser, this yeast possesses spherical cells, from 4 to 6 microns in diameter, which have a thermal death point of 60° C. On liquid media, they produce an adhesive deposit on the walls. The ascospores form at 15° C. at the end of ninety hours. This species ferments saccharose, dextrose and maltose.

CIDER YEASTS OF PEARSE AND BARKER

These species have been isolated by Pearse and Barker [2] from ciders in Alford and Kingston, England.

Yeast A. The cells in beer wort are usually oval while in old cultures they become elongated. The cells developing on gelatin sometimes take the form of sausages. The maximum temperature for budding is situated between 35° and 38° C. Spores are easily formed in 90 hours on potato and porous porcelain at room temperature. They commence toward 15° and stop at 26° C. They are often observed also in old cultures on wort. The ascospores measure 3.1 microns in diameter. At the time of germination the wall of the asc is ruptured, the ascospores swell and germinate by normal budding. On gelatin this yeast forms dry spherical colonies, with slightly indented border. On streaks, it produces a creamy vegetation, with folded, slightly fringed borders. It liquefies gelatin very slowly. This species ferments saccharose, dextrose, levulose and maltose.

Yeast B. This yeast is much like the preceding one in which the cells have the same shape. Their dimensions vary between 6.8 and 10.2 to 4.4 μ. The maximum temperature for budding is around 33° C. Sporulation is accomplished easily on carrot and on porous porcelain. It appears on this last substrate in about 42 hours at 26° and in 90

[1] Kayser, E. Etude sur la fermentation du cidre. Ann. Inst. Pasteur, 4, 1890.

[2] Pearse, B., and Barker, P. The yeast flora of bottled ciders. Jour. Agricultural Science, 3, 1908.

hours at room temperature. It begins at 14° C. It is also observed in old cultures on gelatin. The ascospores are about 3.9 μ in diameter. Germination is accomplished as in Yeast A. This species ferments dextrose, levulose, saccharose and maltose.

Yeast H. This species on beer wort has oval cells, which in old cultures may elongate. The maximum temperature for budding is situated between 30° and 32° C. Sporulation is accomplished easily on porous porcelain, potato, carrot and on wort gelatin. The ascospores are to the number of two, three or four per asc. They have a diameter of 2.3 μ. Their germination seems to be accomplished by parthenogamy. On gelatin, the colonies are white, dry, spherical and on streaks the vegetation is moist with fringed borders. Gelatin is liquefied at the end of some time. This yeast ferments dextrose, levulose, maltose and saccharose.

Yeast I. The cells are oval in the form of a sausage with granules. The maximum temperature for budding is between 35° C. and 38° C. Sporulation is accomplished rapidly on gelatin. On plaster blocks at the end of 22 hours it appears at 26° C. The ascospores measure 3.5 μ in diameter and vary from 2 to 4 per asc. Germination begins by swelling of the ascospore which ruptures the cell wall and normal budding takes place. The colonies on gelatin plates have the appearance of cones with furrows on the surface and fringed edges. On streaks, the vegetation is creamy and moist with irregular borders. This species ferments dextrose, levulose, maltose and saccharose.

Yeast K. This species was found in the black Kingston cider. The cells are oval or sausage shaped. The maximum temperature for budding is around 38° C. Sporulation is accomplished on porous porcelain at 26° C. The ascospores are to the number of 2 to 4 per asc. Their diameter is around 3.9 μ. The colonies on gelatin plates are spherical and dry with thin edges. This species ferments dextrose, levulose, maltose, and saccharose.

SACCHAROMYCES TOKYO. Nakazawa

This yeast was isolated by Nakazawa[1] from the fermentation of Saké. It has spherical cells (1.2 to 3.2 μ) or elliptical cells (3.0–14.0 μ by 2.0 to 9.0 μ) often intermixed with large cells, ovoid or pear shaped. The protoplasm contains few or no granules. The ascospores, of which the number varies from one to four per asc, form in 24 hours at 35° C. The optimum temperature for the formation of ascospores is in the vicinity of 31° C. The ascospore appears in about 16 hours. The minimum is about 10° C. The scum is formed with difficulty. When

[1] Nakazawa, R. Zwei Saccharomyceten aus Sakéhefe. Cent. Bakt. 22, 1909.

cultivated in yeast water containing 5 per cent of saccharose this yeast gives a reddish color. Giant colonies are formed on gelatin wort. *Saccharomyces Tokyo* seems to be a bottom yeast causing rapid fermentation. It ferments dextrose, saccharose, d-galactose and maltose; however it has no action on melibiose nor lactose.

SACCHAROMYCES YEDDO. Nakazawa

This yeast, related to the preceding one, was also isolated by Nakazawa [1] from Saké fermentation. It possesses spherical (3.2 to 6.4 μ) or ellipsoidal cells; often the cells are sausage shaped. Giant cells are often found. The protoplasm is homogeneous and contains few or no granulations. In neutral yeast water, with 5 per cent saccharose, this yeast imparts a yellowish red coloration to the fluid. It produces ascospores to the number of 1 to 4 per asc. The maximum temperature for the formation of ascospores is about 35° C. The ascospores are formed in about 18 hours. The optimum temperature is around 31° C. At this temperature the ascospores appear in about 14 hours. The minimum temperature is between 14° and 10° C. *Saccharomyces Yeddo* forms a thick shiny scum, in which the coloration varies from a white to a yellow. It is a bottom yeast and a slow fermenter. It ferments dextrose, saccharose, d-galactose and maltose but has no action on melibiose, nor lactose.

SACCHAROMYCES FROM SHIRO-KOJI. Saito

This species was isolated by Saito [2] from Shiro-Koji. It possesses globular isolated cells, from 5 to 6 μ in diameter. The contents show a hyaline protoplasm with one or many vacuoles. The ascospores are almost always to the number of two in each asc. They are round and measure 2 to 5 μ in diameter. The giant colonies appear as little points which form a mass of yellow growth without folds. The cultures on gelatin as streaks, produce a liquefaction of this medium. This species never produces a scum on sugar solutions but a ring is secured after fermentation. It ferments dextrose, levulose, d-galactose, saccharose, maltose and raffinose but has no action on melibiose, inuline, lactose and α-methylglucosides. On beer wort, it produces 5.24 per cent of alcohol after 20 days.

[1] Nakazawa, R. Zwei Saccharomyceten aus Sakéhefe. Cent. Bakt. 22, Avt. II, 1909.

[2] Saito, K. Notes on Formosan Fermentation Organisms. The Botanical Magazine, 15, 1902.

SACCHAROMYCES T AND V OF LUDWIG ROSE [1]

These species were isolated from the mucous secretions of two oaks in which they were found associated with *Saccharomyces Ludwigii*, *Saccharomyces apiculatus*, Yeasts F and G of Rose, and *End. Magnusii*. They have the same characteristics and are apparently identical. Their cells are elliptical, later becoming round. Their diameter is about 5.5 μ. These two yeasts form ascospores easily to the number of two or four in each asc. The optimum temperature for the formation of ascospores on plaster blocks is 25° C. These species seem to resemble yeast No. 689, isolated by Lindner [2] from secretions of trees in the Berlin botanical garden. In wort they produce an active fermentation of the bottom type. They ferment dextrose, d-mannose, d-galactose, levulose, saccharose, maltose, raffinose and α-methylglucoside.

B. Second Sub-Group

Yeasts fermenting dextrose and saccharose but having no action on maltose or lactose.

SACCHAROMYCES MARXIANUS. Hansen

This species was found by Marx on grapes and described by Hansen.[3] In must it produces small oval cells which resemble very much *S. exiguus* and *S. ellipsoideus* (Fig. 118). However, they are easily distinguished from these two yeasts by the fact that they form colonies of long cells, very rapidly, in the shape of a sausage, and later on flocks which float on the liquid. These are composed of cells having the appearance of mycelium and resemble the formations which one observes in scums of certain other yeasts (*S. cerevisiae, Pastorianus* and *ellipsoideus*). These colonies are formed of cells which are easily detached from their point of connection. On gelatin the cells develop with a true mycelial formation with cross walls resembling the mycelium of *Monilia candida* (Fig. 119).

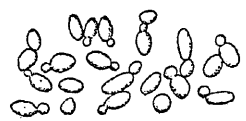


Fig. 118. — *Saccharomyces Marxianus*. Cells from Sediment after One Day in Beer Wort (after Hansen).

[1] Rose, L. Beitrage zur Kenntniss der Organismen in Eichenschleimfluss. Inaugural Dissertation, University of Berlin, June 25, 1910.

[2] Lindner, P. Mikroskopische Betriebskontrolle in der Gärungswerben. Paul Parey, Berlin, 6th edition, 1909.

[3] Hansen, E. Ch. Recherches sur la physiologie et la morphologie des ferments alcooliques. VII. Action des ferments alcooliques sur les diverses espèces de sucre. Levures alcooliques à cellules ressemblant à des Saccharomyces. C. R. du lab. de Carlsb. v. II, Book 5, 1888; C. R. du lab. de Carlsb. v. V, Book 2, 1902.

The temperature limits for budding on beer wort are: minimum 0.5° C. and maximum 46–47° C. The maximum temperature of sporulation is situated between 32 and 34°, the minimum temperature being between 4 and 8°. The optimum is between 22 and 25° C. (Klöcker [1]). Sporulation is effected very easily and most abundantly in yeast water with 10% of must, and on plaster blocks. The ascospores are spherical or oval and measure 3.5 μ in diameter.

This species produces only traces of a scum which appears only after two or three months of culturing on must. Its scum is made up of ellipsoidal cells with a few elongated and sausage shaped.

In beer wort this produces after a long time from 1 to 3% of alcohol by volume.

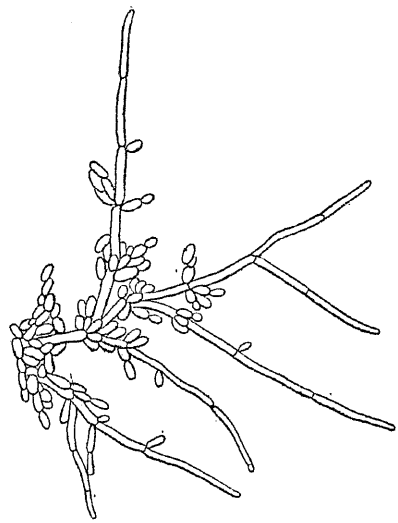


Fig. 119. — *Saccharomyces Marxianus.* Formation of Mycelium in Gelatin to which Yeast Water Has Been Added (after Hansen).

It inverts and ferments saccharose. It also ferments dextrose, d-mannose, d-galactose, levulose, raffinose and inuline, but it does not act on melibiose.

SACCHAROMYCES MANDSHURICUS. Saito [2]

Saito isolated this yeast from Chinese yeast used in the making of Sorgho, an alcoholic drink of Manchuria. He isolated *Saccharomyces*

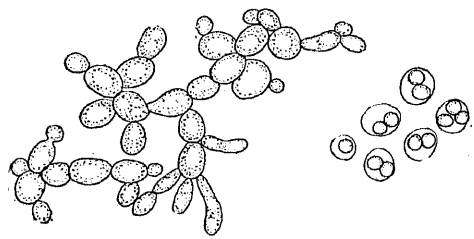


Fig. 119–A. — *Saccharomyces mandshuricus.* Cells from Sediment in Wort at 28° C.; An Asporogenic Species (Saito).

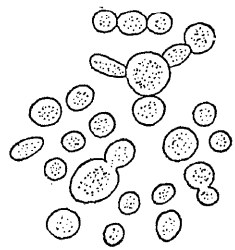


Fig. 119–B. — *Saccharomyces mandshuricus.* Cells from Sediment in Beer Wort at 28° C. (Saito).

[1] Klöcker, A. Rech. sur les S. Marxianus, apiculatus et anomalus. C. R. des trav. du lab. de Carlsberg, v. IV, 1895.

[2] See reference for *Zygosaccharomyces Mandshuricus.*

mandshuricus I, II, III, and *IV.* The cells are oval or globular (6–8) in diameter. On gelatin large white round colonies are obtained. In their centers, there is a sort of crater with canals running out around the periphery. On beer wort, a scum is formed after a time. The spores are globular (2.7–4) and on Gorodkowa's gelatin medium they germinate by ordinary budding. The temperature limits for sporulation are 11° C. and 38° C. This yeast ferments levulose, dextrose, mannose, galactose, maltose, saccharose and raffinose. *Saccharomyces mandshuricus* II, III and IV are very closely related to this species.

SACCHAROMYCES EXIGUUS. Reess-Hansen

This species was found by Hansen[1] in pressed yeast. It develops in must with a vegetation resembling *S. exiguus* described by Reess. It is not possible to separate it with a certainty from this latter yeast.

The cells are small and resemble the cells of *S. Marxianus,* but they never give the mycelial formation. They do not contain glycogen.

The formation of ascospores and scums is not very abundant. On the contrary, this yeast forms rings on the culture tube. The cells of the scum resemble those of the sediment. However, small cells and short, tubular forms are much more frequent.

When cultivated in must this *S. exiguus* produces only feeble quantities of alcohol. It does not cause any disease in beer. It ferments saccharose, dextrose, levulose, raffinose, dextrin and sometimes inuline, but it does not act on maltose and d-mannose or melibiose.

SACCHAROMYCES ZOPFII. Artari

This yeast was found during the manufacture of sugar in Saxony. Since that time it has been found by others in samples of syrup in the making of sugar. Owen[2] stated that this yeast was the principal agent causing a deterioration of the product. Browne isolated several varieties of yeasts from Cuban raw sugar. These are described elsewhere.



Fig. 120. — *Saccharomyces Zopfii* (after Lindner).

[1] Hansen, E. C. Recherches sur la physiologie et la morphologie des ferments alcooliques. VII. Action des ferments alcooliques sur les diverses espèces de sucre. Levures alcooliques à cellules ressemblant à des Saccharomyces. C. R. du lab. de Carlsb., v. II, Book 5, 1888; C. R. du lab. de Carlsb., v. V, Book 2, 1902.

[2] Owen. The occurrence of Saccharomyces Zopfii in cane syrups and variation in the resistance to high temperatures when grown in solutions of varying density. Cent. Bakt. Abt. II, 39, 1913.

This yeast has been isolated from the manufacture of sugar in Saxony.[1] It is composed of short ellipsoidal or spherical cells in which the diameter may reach from 3 to 6 μ, exceptionally 8 μ (Fig. 120). When the species is cultivated in a solution of dextrose with 5 to 8% ammonium sulphate added, it produces walled cells. The maximum temperature for budding in must is 33–34° C., the optimum is 28–29° C. Sporulation is easily accomplished as well on liquid media as on solid media. The maximum temperature for the formation of ascs is about 32 and 29° C. Ascospores commence to appear at this temperature at the end of 21 hours. The ascospores are spherical and measure from 1.5 to 3 μ in diameter. Their number is ordinarily two per asc, but it may vary from one to four. The vegetative cells are able to resist a temperature of 130° dry heat for a half hour, and from 66–67° moist heat. According to Owen this yeast is able to resist 90° C. for 10 minutes which would locate the thermal death point at 90°.

SACCHAROMYCES COREANUS. Saito

This species was isolated by Saito[2] from Koji from Korea. The cells are spherical, oval or sausage shaped, and possess a very resistant wall. Their average dimension is from 3–7 μ. The contents are homogeneous and hyaline, sometimes with large vacuoles. The cells dissociate rapidly in such a way that they often appear isolated.

This yeast forms ascospores very easily on plaster blocks. The temperature limits for sporulation are: minimum 18–20°; optimum 31–34°; and maximum toward 35–36° C.

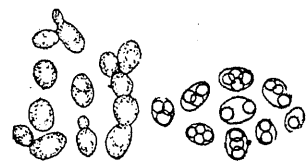


Fig. 121. — *Saccharomyces, forma major. a,* Young Vegetation from Sediment; *b,* Ascs (after Saito).

Each asc possesses from one to four ascospores, most of them having two to four (2 to 3.5 μ in diameter). The ascospores germinate by ordinary budding.

S. coreanus produces a moist scum in sugar solutions at 25°. It ferments dextrose, levulose, saccharose, d-galactose, melibiose and raffinose, but has no action on maltose, lactose, arabinose, inuline or dextrin.

Giant colonies develop on decoction of Koji gelatin and have a

[1] Artari, A. Ueber einen im Safte d. Zuckerfabriken in Gemeinschaft mit Leuconostoc schädlich auftretenden, den Zucker zu Alkohol u. Säure vergärenden Saccharomyces (S. zopfii). Abh. d. naturf. ges. zu Halle, v. XXI, 1897.

[2] Saito, K. Notizen über einige Koreanische Gärungsorganismen. Centr. f. Bak., v. XXVI, 1910.

grayish white color. The center is slightly concave with a surrounding surface of radial bands. The edge is very much indented. On Koji gelatin in plates, the colonies are punctiform moist with a grayish-white color. The gelatin is not liquefied. In streaks on the same medium this yeast furnishes a thick white growth with an indented border.

SACCHAROMYCES COREANUS. *Forma Major.* Saito

Isolated under the same conditions as the preceding one, this yeast is scarcely distinguishable by its dimensions, which are, however, a little larger. The cells are spherical or oval (8 to 12 μ in diameter) (Fig. 121, a) and cause the formation sometimes of short mycelial formations. The ascospores are 2 to 4 μ in diameter (Fig. 121, b). This yeast forms no scum on a decoction of Koji.

SACCHAROMYCES JÖRGENSENII. Lasché

This yeast was described by Lasché.[1] It possesses small round or oval cells (2.5 to 5 μ). The optimum temperature for sporulation is 25° C.; the temperature limits are 8°–12° and 26°–30°. At this higher temperature, vegetation rapidly disappears. The ascospores are spherical and very refractive. They are present ordinarily to the number of 2 or 3, rarely 4, per asc. No scum formation has been noticed. In old cultures, one may observe a scant ring formation resembling that of a brewery yeast. Gelatin is slowly liquefied. Cultures on streaks have a gray color with a regular edge.

C. Third Sub-Group

Yeasts fermenting dextrose and maltose but having no action on saccharose or lactose.

SACCHAROMYCES ROUXII. Boutroux[2]

This yeast was discovered in the juice of certain fruits. Its cells are small, 4 to 5 μ in diameter. They are spherical or ellipsoidal and often arranged in chains. The ascs contain from 1 to 3 ascospores. They are produced especially on nutrient fluids. This species produces no extended scum but simply small floating islands.

[1] Lasché, A. Saccharomyces Jörgensenii. Der Braumeister, Chicago, 1892, and Zeitschr. f. d. ges. Brauw. 15, 1892.

[2] Boutroux, L. Sur l'habitat et la conversation des levures spontanées. Bull. de la soc. Linn. de Normandie, Vol. VII, 1883; Ann. de l. sc. nat. Botan. 17, 1884.

YEAST FROM PULQUE NO. 2. Guilliermond [1]

This yeast was isolated from the fermentation of Pulque, an alcoholic drink prepared in Mexico. In beer wort it develops as a very abundant sediment with a whitish yellow color. A wine fermentation is produced in beer wort and a very evident cloudiness. After a certain time, it forms small flocs with a sparkling appearance which float in the medium. Ring or scum formations have not been observed. The sediment is made up of cells with variable shapes, either oval, round, or elongated; with pointed ends which resemble somewhat *S. Ludwigii*. In general, budding is accomplished at both ends of the cells. Often many buds are formed at the same time at each end, as in the yeast-like structures of the *Dematium*. Even round cells budding like *Torula* may also be observed. After a few days there is a production of a deposit in the flask and a true mycelium which buds like a *Monilia*. The maximum temperature for growth is situated near 40° C. and the optimum near 29°–30° C. The spores are formed very easily and abundantly on most solid media. They appear not only in the yeast-like cells but also in cells in the mycelium. Generally, they are to the number of four per cell. The maximum temperature for sporulation is near 37° C., the optimum near 25° C.

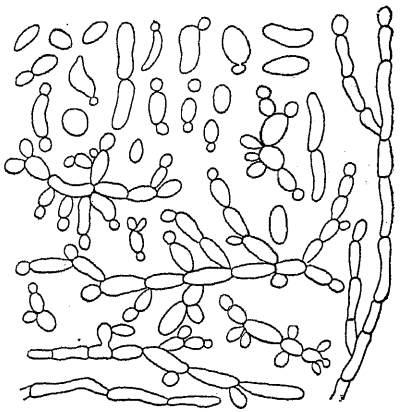


Fig. 121–A. — Yeast from Pulque No. 2. Sediment in Beer Wort after 15 Days at 30° C. (after Guilliermond).

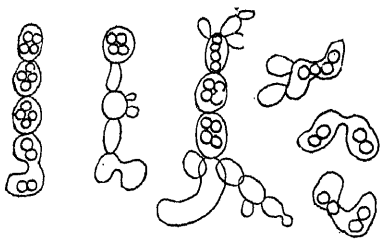


Fig. 121–B. — Copulated Ascospores Changing into Asci after having Developed Cells in Yeast from Pulque No. 2.

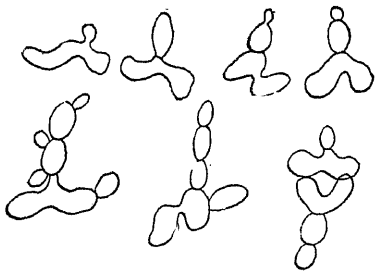


Fig. 121–C. — Copulation of Ascospores and Their Germination in Yeast from Pulque No. 2 (after Guilliermond).

[1] Guilliermond, A. Levaduras del Pulque. Boletín de la Dirección de Estudios Biológicos, Mexico, 1917.

Germination of the spores is always similar to that in *S. Ludwigii*. The spores fuse two by two by a copulation canal and later germinate by ordinary budding. It often happens, in unfavorable solid media, that the spores after having united two by two, change immediately into normal ascs

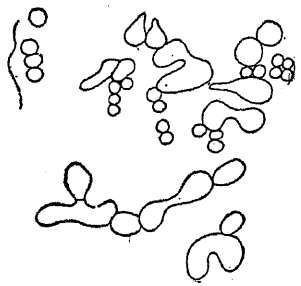


Fig. 121–D. — Germination and Copulation of Spores in Yeast from Pulque No. 2.

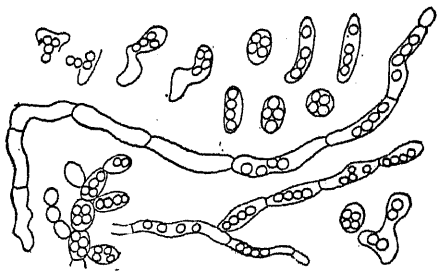


Fig. 121–E. — Ascs from Yeast from Pulque No. 2 on Beer Wort Agar at 30° C.

This yeast inverts and ferments saccharose. It is curious and aberrant having certain analogies, in the formation of its mycelium and other characteristics, to *Saccharomyces Ludwigii*.

ZYGOSACCHAROMYCES SOJA. Takahashi and M. Yukawa [1]

Syn.: SACCHAROMYCES SOJA. Saito [2]

This yeast was isolated during the early stages of ripening of "Shoju Moromi," and seems to be an important species for "Shoju" manufacture. Excepting the fermentability of galactose, *Saccharomyces soja* seems to be similar to this yeast; moreover, there is not a great difference between *Torula "Shoju"* and this yeast. According to Saito's illustration it is questionable that he, who gave the name of *Zygosaccharomyces japonicus* to this "Shoju" film yeast, comprised his "Shoju" yeast into the genus of *Saccharomyces*. Jörgensen [3] also has the same inference about this question.

In "Koji" extract or wort after 5 days at 20° C. the young cells are commonly spherical or oval, 3.5–8 μ in diameter. The contents are homogeneous and sometimes exhibit vacuoles, and are rich in glycogen. The cells of old cultures in "Koji" extract or wort after

[1] Takahashi, and Yukawa M. Original communications, Eighth Internatl. Congress of Applied Chemistry, v. XIV, 1912, p. 166.

[2] Saito, K. Cent. f. Bak. II, Ab. XVII, 1906.

[3] Jörgensen. Die Microorganismen d. Gärungsindustrie. IV. Aufl. Jörgensen, 370.

2–6 months have already been described, and are almost the same as in *Zygosaccharomyces major*.

On "Koji"-extract-gelatin-plate this yeast forms bright pearly, grayish white, mostly round, and elevated colonies. On "Koji"-extract agar streak at 27° C. it forms a grayish white, waxy, elevated surface, but after a month it becomes somewhat brownish and the center of the growth becomes flat. The edge shows tooth-like engravings. In glucose "Saké" agar the growth is yellowish white, of waxy luster, and forms an elevated smooth surface with fine streaming lines. The edge is somewhat uneven. On stab the growth is the same as with the preceding species, but the surface of the isle is more concentrical. In fluid culture the appearance of development of this species is very similar to that of *Zygosaccharomyces major*. This species can also reproduce and ferment in every nutrient fluid which contains 20% NaCl.

This yeast ferments dextrose, levulose, maltose, mannose, but does not ferment saccharose, raffinose, galactose, lactose, α-methylglucoside.

Formation and germination of spores in this species have already been described fully. The form and size of spores are similar to those of *Zygosaccharomyces major*, but the numbers of sporogenic cells are always less than in the latter species. Moreover, the time required for the occurrence of sporulation is longer than that of *Zygosaccharomyces major*.

This species does not ferment saccharose but *Zygosaccharomyces major* attacks the same sugar quickly and both species are easily distinguished from each other by dimensions of the cells and the growths on glucose-"Saké"-agar.

This species differs from *Zygosaccharomyces Barkeri* by the sporogenic point of view and the behavior toward maltose, and from *Zygosaccharomyces priorianus* by the cell forms of young cultures and the circumstance of sporulation. *Zygosaccharomyces javanicus* is easily distinguished from our yeast by the size of cell and the fermentability of galactose, and the formation of large numbers of spores on agar. *Zygosaccharomyces lactis* α ferments lactose but not maltose. *Zygosaccharomyces japanicus* produces easily a particular film on the surface of nutrient fluid. Both *Zygosaccharomyces fusoriens* and *Zygosaccharomyces from cocoa* do not ferment saccharose as does *Zygosaccharomyces soja*, but both species ferment dextrine strongly.

On the other hand, *Saccharomyces soja* and *Torula "Shoju"* seem to stand in close relation to *Z. soja* yeast; however, it might be appropriate to group these three yeasts together into one and the same species. Be that as it may, we will give it the name of *Zygosaccharomyces soja*.

SACCHAROMYCES LINDNERI. Guilliermond [1]

This yeast was isolated from a ginger alcoholic drink which is quite similar to the English ginger beer. On beer wort, at 25° C., the yeast develops at the bottom of the flask in the form of a white sediment. The cells are oval or ovoid, rarely round, like those of *Saccharomyces ellipsoideus*. The yeast then belongs to the *ellipsoideus* type. The cells have an average dimension of 5.2 μ in length and 4.5 μ in width. After three months the cells in the sediment take on a peculiar appearance. The growth is vigorous and includes a large number of giant cells, round or elongated, often in the shape of a curl. In old cultures, the cells tend to take on the round shape. The temperature limits for budding on beer wort are: minimum, below 5° C., maximum, 40–41° C. Near the temperature limits, the cells have the same shape as at other temperatures. On beer wort at 25° C., this yeast forms a feeble ring after 12 days but never produces a scum. Sporulation is accomplished easily on slices of carrot, Gorodkowa's gelatin medium and plaster blocks. When cultivated for a long time on agar it loses slowly its sporogenic functions as happens in many other yeasts (Lindner). The temperature limits for sporulation have not been given careful study. The spores are to the number of from 1 to 4 per asc. They are spherical and have a diameter of 2 to 3 μ. Germination is accomplished exactly as in *Saccharomyces chevalieri* and *Mangini*. It is generally preceded by a copulation of spores. On agar streaks at 25° C., there is produced a grayish white growth. After 15 days and up to two months, the colony has the appearance of a damp white layer. The center is a little raised and includes a number of marked raised portions. The periphery is transparent and is characterized by a number of jutting-out canals. The edge is undulated. Stab cultures in wort agar give a funnel shaped growth after 25° C. The giant colony on wort agar at 25° C., after 15 days, is well developed with a white, slightly yellowish, color.

This yeast causes an active fermentation in beer wort. It ferments saccharose, levulose, and d-mannose, and dextrose a little, but has no action on d-galactose, lactose, dextrine and maltose.

SACCHAROMYCES PARADOXUS. Batschniskaia

This species was isolated from the mucous secretions of trees at Petrograd. The cells measure 3.6–7.2 × 2.6–6 μ. They possess a rather special form of development, the interpretation of which is

[1] Guilliermond, A. Monographie de levures rapportées d'Afrique occidentale par la Mission Chevalier. Annales des Sciences Naturelles Botaniques, 19, 1914.

difficult. From 1 to 8 ascospores are formed in each asc, generally 4. Germination is preceded by a fusion of the ascospores. This is accomplished between two ascospores but usually one may see from one to three or a greater number fusing. The cells which result from this fusion finally elongate and take on various shapes, giving a sort of promycelium. In this promycelium, there are formed many generations of buds. The cells which result from the budding fuse two by two, and these are the cells, which by budding, produce vegetative cells. Streaks on gelatin give colonies which are white, small and striking. On must agar, the yeast develops under the form of a brilliant coating, viscous and light yellow. The giant colonies on must agar have the same color as the colonies on streak cultures. Bouillon cultures with beer wort added give no scum but a brown sediment and a cloudiness. This yeast ferments glucose, levulose, saccharose and galactose. A cytological study of the yeast seems advisable in order to interpret the cell structure during the different stages of growth.

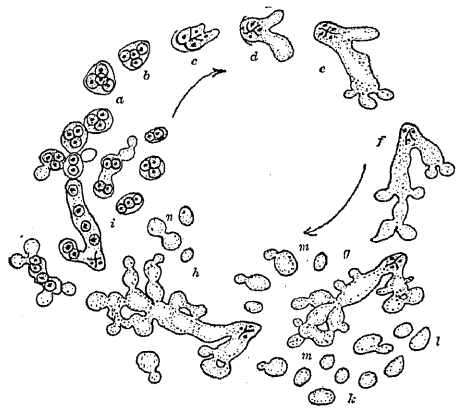


Fig. 121–F. — *Saccharomyces paradoxus.* *a* and *b*, Ascs; *c*, *d*, *e*, and *f*, Fusion of Cells and Formation of Promycelium; *m*, *g*, *l*, yeast Cells Derived from Budding of Copulated Cells Formed by the Promycelium; *i*, Promycelium Transforming Directly into an Asc (after Batschinskaia).

SACCHAROMYCES MANGINI. Guilliermond [1]

This yeast was isolated from fermenting wine Bili made at Conakry and was found along with a species named *Zygosaccharomyces Chevalieri*. This wine is a drink prepared from tubercles of the *Osbeckia grandiflora*.

On beer wort at 25° C., *S. Mangini* forms an abundant white sediment. When examined microscopically after 24 hours the sediment seems to be made up of oval or round cells resembling somewhat those of *S. ellipsoideus*. This yeast then also belongs to the *ellipsoideus* type. The cells are isolated and sometimes united into budding colonies of from 2–4 cells. They are smaller than the cells of *S. Chevalieri*. The average dimensions are about 4.4 μ wide and 6.75 μ in length. The cells keep the same form after 15 days.

[1] Guilliermond, A. Monographie des levures rapportées d'Afrique occidentale par la Mission Chevalier. Annales des Sciences Naturelles Botaniques, 19, 1914.

The temperature limits for budding on beer wort are a minimum below 5° and a maximum of 40–41°. The shape of the cell is the same at the temperature limits as at the optimum temperature. A feeble ring but no scum is formed at the end of 11 days.

Sporulation is easily accomplished on slices of carrot and Gorodkowa's gelatin and the plaster block. The ascs contain from 1–4 spherical spores, 2–2.5 μ in diameter. Spores germinate exactly as those of *S. Chevalieri*. On wort agar at 25° the yeast develops after three or four days with a train of white, moist colonies. The center is slightly indented and the border slightly sinuous. The center is thick and granular, the periphery surrounded by a number of canals running out from the center.

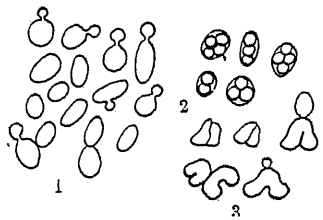


Fig. 121–G. — *Saccharomyces mangini.*

1. Cells from Sediment in Beer Wort for 24 Hours at 25°C. — 2. Ascs. — 3, Germination of Ascospores.

On wort agar stab cultures at the end of 15 days at 25° the colony is funnel shaped. On wort gelatin at 20° the colony is round with a slightly raised center. There is no liquefaction of gelatin. The yeast has the characteristics of a top yeast and causes a rather active fermentation of beer wort. It ferments saccharose, dextrose, levulose, d-mannose, lactose, d-galactose, and dextrine.

SACCHAROMYCES CHEVALIERI. Guilliermond [1]

This yeast was isolated in a wine fermentation from the Ivory Coast. On beer wort at 25°, this yeast forms an abundant white sediment. When examined at the end of 24 hours, this sediment shows large cells, spherical or oval in shape. Many give birth to long buds sometimes in the form of sausages. A certain number of the cells are elongated, but the round or oval cells are the most frequent. The cells of this yeast belong to the *ellipsoideus* type.

The dimensions of the cells vary between 5 and 9 μ in length and 4 and 7 μ in width. The average dimensions are about 5.53 μ long and 4.14 μ wide. The cells are frequently united in small colonies of about 3–10 budding units. In older cells these colonies are generally spherical or oval, while the young cells have a tendency to elongate.

The temperature limits for budding on beer wort are a minimum below 5° C. and a maximum of 40–41° C. Near these temperature limits the yeast has the same cellular forms as at other temperatures.

[1] Guilliermond, A. Monographie des levures rapportées d'Afrique occidentale par la Mission Chevalier. Annales des Sciences Naturelles Botaniques, 19, 1914.

A feeble ring is formed at 25–30° on beer wort after about 12 days. This ring is made up of spherical or oval cells united in groups. The mycelial formation has not been observed.

Spores are formed quickly on slices of carrot, Gorodkowa's medium and the plaster block. The temperature limits on plaster block are maximum 39–40° and minimum 8–10°. The optimum is situated at about 25–30°. At this temperature the spores appear in about 12 hours. The spores are to a number of from 1 to 4 per asc. They are spherical and have a diameter of from 2.5 to 3.5 microns.

Germination is generally preceded by sexual processes analogous to those which have been described for Johannesburg II yeast. At the beginning of germination the spores enlarge; the wall of the asc disappears, but may persist during the first stages of germination. About one-fourth of the spores germinate only by ordinary budding without preliminary copulation. The others unite two by two by means of a copulation canal.

On wort agar in streaks *S. Chevalieri* produces at the end of three days a train of grayish white growth with a slightly indented border; at the end of 15 days to a month the colony is white with a damp appearance; its center is thick and border slightly undulated. On wort gelatin in stab cultures the yeast develops a colony which is funnel shaped. The surface has a damp appearance; it is thick at the center and thin at the edges. The giant colony on wort agar at the end of 15 days at 25° is well developed, spherical, slightly moist, and has a grayish white color. It is made up of a central granular portion and a thin transparent peripheral part.

S. Chevalieri has the characteristics of a top yeast. It causes a rather active fermentation on beer wort. It ferments saccharose, dextrose, levulose and d-mannose quite actively, but does not seem to have any action on galactose, maltose or lactose.

SACCHAROMYCES ETIENNE. Potron [1]

This yeast was isolated from sputum from a disease in which it was the causal agent. The infection began with a gastro-enteritis which later turned into a pleuro-pulmonary trouble which had some of the appearances of tuberculosis. The sickness yielded to treatment with iodine. The yeast develops on carrot and potato. It has cells which vary from spherical to ellipsoidal in shape (3–9 μ long and 4–5 μ wide). Curled cells are more numerous in scums and old cultures. The ascospores appear on carrot after 30 hours. The

[1] Potron. Presence d'une levure au course d'une infection pleuropulmonaire grave. Soc. de Méd. de Nancy, 1914.

asces usually contain 4 elliptical ascospores (2-2.5 μ). The wall of the asc is broken when the spores germinate. Germination is by ordinary budding. On potato at 25° C., punctiform spherical colonies are formed having a grayish white color. These become confluent into large colonies with festooned edges. White confluent colonies develop on carrot. The yeast ferments glucose.

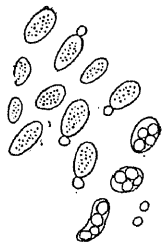


Fig. 121–H. — *Saccharomyces Etiennei* (after Potron).

D. Fourth Sub-Group

Yeasts fermenting dextrose, but having no action on saccharose, maltose or lactose.

SACCHAROMYCES MALI DUCLAUXI. Kayser

This species was found by Kayser [1] in a sample of cider. The cells are large (6 to 12 μ long and 4 to 7 μ wide) and form a light floating growth. They are killed at 55° C. and are very sensitive to acids. Ascospores are formed at the end of 30 hours at 15° C. This yeast acts on neither saccharose nor maltose but ferments invert sugar imparting a " bouquet " to the solutions.

SACCHAROMYCES UNISPORUS. Jörgensen [2]

This yeast was discovered by Holm. By its action on sugars, it is related to *Saccharomyces mali Duclauxi*. The cells are small and oval. In old cultures, one may see the shape of the cells of *Pastorianus*. The spores appear at the end of 40 hours at 25° C. After 72 hours at 15° C. only a few ascs are visible. The ascospores are round and refractive. This yeast does not form a scum but simply a ring in old cultures.

E. Fifth Sub-Group

Yeasts which ferment lactose.

SACCHAROMYCES FRAGILIS. Jörgensen [3]

This yeast was encountered in kefir, an alcoholic milk produced by the fermentation of *S. fragilis* and many Torula and many bacteria among which is *Bacillus caucasicus*. *Saccharomyces fragilis* pos-

[1] Kayser, E. Études sur la fermentation du cidre. Ann. Past. Inst. 4, 1890.

[2] Jörgensen, A. Die Mikroorganismen der Gärungsindustrie, Berlin, 5th edition, Paul Parey, 1909.

[3] Jörgensen, A. See reference for S. unisporus.

sesses small oval or elongated cells. (Fig. 122, A.) The temperature optimum for budding is towards 30° C. Asci are produced on plaster blocks at 25° C. in twenty hours, and at 15° C. in forty hours. They are also formed in fermenting solutions and on gelatin. The ascospores are round or elongated (Fig. 122, B). After a long time, this



A B

Fig. 122. — *Saccharomyces fragilis.* A, Young Vegetation on Yeast Water with Added Lactose. B, Asci (after Holm).

yeast produces a scant scum. At room temperature this yeast forms about 1 per cent of alcohol by volume after eight days and 4 per cent after 4 months. In beer wort, after six days, about 1 per cent of alcohol is formed. According to Bau this yeast ferments lactose, but has no action on melibiose.

SACCHAROMYCES FLAVA LACTIS. Krueger [1]

This species is found in beer to which it contributes an abnormal yellow color, and a disagreeable odor, resembling putrefied urine. It possesses small cells attached in chain formation. They sporulate rapidly on slices of carrot. The colonies on gelatin are yellow. Gelatin is liquefied very rapidly and the colonies cover themselves with a scum. This yeast produces a yellow scum in milk and in a decoction of milk sugar. The coloring matter is formed only in contact with air.

SACCHAROMYCES ACIDI LACTICI. Grotenfelt

Grotenfelt [2] has described under this name a yeast which, introduced into sterile milk, causes a coagulation and at the same time a formation of acid. Its cells are ellipsoidal (2.0–4.35 μ long and 1.5–2.9 μ wide). On gelatin, and on agar, it forms white shiny colonies. On potato it forms large moist spots clear gray which turn to a brown.

[1] Krueger, R. Bakter. chemische Untersuchungen käsiger Butter. Cent. Bakt. 7, 1890.

[2] Grotenfelt, G. Ueber die Spaltung von Milchzucker durch Sprosspilze und über schwarzen Käse. Fortschr. der Med. 7, 1889.

It ferments lactose giving 0.108 per cent of alcohol in eight days. Grotenfelt pretends to have obtained ascospores on potato but the existence of these ascospores does not seem to be well established. Then, again, it is possible that this species may be a *Torula*.

SACCHAROMYCES LACTIS *a*. Dombrowski [1]

This species, isolated from Bulgarian yoghourt, has been described in the laboratory of Professor Jensen at Copenhagen by Dombrowski. Generally it is a yeast with elliptical cells but it may present cells elongated to 18 μ especially on solid media. On grape must, the cells may be 9.0–6.0 μ long and 3.75–3.25–3.00 μ wide. In cultures on the cover glass the formation of giant colonies is easily observed.

Sporulation is difficult to obtain especially after successive culturing in liquid media. Ascospores are formed at the end of about 44 hours at 25° C. in cells arising from solid media. The ascospores are round and to the number of three to four in each asc. They germinate by ordinary budding.

On beer must gelatin in plates, the colonies are lenticular. In stabs, the growth is along the line of inoculation as one approaches the surface. Giant colonies on beer must gelatin, after two months, offer a delicate structure with concentric zones and rays running out from the center.

Saccharomyces lactis α acts like a bottom yeast in sugar solutions. It never produces a scum, but a ring is formed at the end of six weeks at room temperature. In beer must, it produces a fermentation accompanied by the formation of an agreeable odor. It decolorizes the wort in 10 days and yields after $4\frac{1}{2}$ months four to five grams of alcohol per 100 c.c. It causes an active fermentation in milk at 23–25° C. It ferments lactose, saccharose and dextrose but does not act on maltose. A small amount of acids are found among the products of the fermentation.

SACCHAROMYCES LACTIS β. Dombrowski [1]

This species has been isolated by Dombrowski from a sample of milk fermented at 45° C. The cells possess variable forms. One may find egg-shaped or elliptical cells aside from the elongated cells which may reach 20 microns. These latter cells remain attached and form long chains or a sort of mycelium. On beer wort, the cells measure 7.6–5.5–4.6–3.8 μ in length and 4.6–3.8 μ in width. Sporulation is ac-

[1] Dombrowski, W. Die Hefen in Milch und Milchprodukten. Cent. Bakt. 28, 1909.

complished easily. They appear at the end of five hours at 25° C. on plaster blocks. They may also be observed in cultures on gelatin, in hanging drops and in the scums of old cultures on liquid media. The ascospores are to the number of from one to eight per asc. Their form and dimensions are variable. More often they are elliptical but sometimes they are hemispherical. They germinate by ordinary budding after absorbing the wall of the asc. The colonies on nutrient agar, in plates, are circular and torpedo shaped. In stabs, the growth is along the line of inoculation and increases towards the surface. Giant colonies show a center with a crateriform concavity with radii out from the center. *Saccharomyces lactis* β produces in liquid media with sugars, a scum and a ring in which the cells have somewhat the form of a mycelium with ascospores. It acts as a bottom yeast. An active fermentation is produced during which a feeble aroma may be noticed. Beer wort is distinctly decolorized and there is formed at the end of five and a half months 7.93 per cent of alcohol by volume. In milk an energetic fermentation is produced at 23–25° C. It ferments saccharose, lactose, d-galactose and dextrose but has no action on maltose. It seems to be closely related to *Saccharomyces fragilis* (Jörgensen).

YEAST FROM KOUMYS. Schipin

This yeast was isolated from koumys by Schipin. Along with a few bacteria, it contributes to the formation of koumys by inducing a fermentation in milk with a small quantity of lactic acid. Rubinsky [1] has given a detailed description of this organism. In hanging drops this yeast possesses round or oval forms which contain at one of their extremities or in the middle a large refractive granule. In old cultures on agar, in Petri dishes (after four or five weeks) the cells are often elongated. Old cells always contain granules.

Schipin has obtained sporulation in this yeast. Rubinsky has also observed on plaster blocks the formation of six or eight globules which look like ascospores but it is not certain that they are true ascospores. On gelatin plates, this koumys yeast exhibits mediocre development during the first few days. At the end of three days, it forms surface colonies of about 2–3.5 millimeters and the deep colonies are round. The culture gives off an aromatic odor often acid. On gelatin stabs, the culture takes the form of a bottle and has a flat white color sometimes yellow along the line of puncture. On gelatin added to bouillon, after 2 to 3 weeks, the colonies have a center sometimes soft and shiny. In cabbage bouillon, the colonies have a peculiar appearance. The

[1] Rubinsky, B. Studien über den Koumiss. Cent. Bakt. 28, 1910.

middle is thin, dull, finely granular and dry. The edge is distinctly raised above the central part surrounded by a granular portion.

The colonies on gelatin possess no characteristic appearance. The yeast gives a growth resembling a string of pearls. The cultures on gelatin after from 3 to 5 weeks show a liquefaction. At 37° the culture is juicy, shiny and white. It forms great white colonies. Later they spread all over the surface.

In milk at 37° C. the koumys yeast produces a strong fermentation of the lactose and yields about 36 per cent of lactic acid. The liquid is cloudy at first but clears up showing an abundant vegetation in the sediment of a varying white or yellow color. It never produces a scum. It has the characteristics of a bottom yeast. It decomposes the casein which it changes to albumoses and peptones and produces aromatic ethereal substances which impart to koumys its aroma.

SACCHAROMYCES ANAMENSIS. Will [1]

This yeast, which has been employed in distilleries under the name of "Levure anamite," is a top yeast of the wild variety. The cells are generally oval, sometimes elongated (4 to 9 μ). Giant cells may be noticed in cultures. Groups are rarely formed by individual cells. The spherical ascospores, one to four per asc (2.4 to 4 μ) have an optimum temperature for sporulation of 33° C.; a maximum of 35° C. and a minimum of 12° C. This yeast forms a scum made up of round or oval cells, sometimes with cells shaped like a sausage. The optimum temperature for the formation of scums on beer wort is near 31° C. This yeast ferments dextrose, levulose, galactose, saccharose, maltose and raffinose. Lactose is assimilated, but the yeast is able to ferment it but feebly.

SACCHAROMYCES TAETTE, *Major* and *Minor* Olsen-Sopp [2]

These two yeasts were isolated from Taette, a milk used in times of antiquity by the people of the North (Norway and Sweden). It is a thick viscous milk, not coagulated, but with an acid taste which is quite agreeable. *Saccharomyces taette major* is distinguished from the second variety by the fact that its cells are much larger and that it produces ascospores. The second type, *Saccharomyces taette minor*, does not give ascospores. Taette does not contain over 0.3

[1] Will, H. Saccharomyces anamensis, die Hefe des neuen Amyloverfahrens. Cent. Bakt. Abt. 2, 39 (1913) 26.

[2] Olsen-Sopp, O. J. Taette, the primitive Norse storage milk and associated milks; their significance as a nutrient. Cent. Bakt. Abt. 2, 33 (1912), 1–5.

to 0.5 per cent of alcohol by volume which results from a fermentation of the lactose by these two species.

F. Sixth Sub-Group

Yeasts which do not produce alcohol and in which the characteristics of fermentation are little known.

SACCHAROMYCES CONGLOMERATUS. Reess

This yeast was found by Reess [1] on decayed grapes and at the beginning of the wine fermentation. The description of the author is reproduced here. "Budding cells, round, 5 to 6 μ in diameter united in bunches. This formation is accomplished in the following manner. On the axis of two old cells, before they germinate, by budding in the direction of their longitudinal axis there are formed simultaneously many buds which branch out. The asci are frequently united two by two in each cell. Two to four spores are present which during germination give rise to new bunches."

Hansen has never observed a yeast which may be compared to *Saccharomyces conglomeratus*. He thinks that this yeast may not be a well-differentiated yeast but may be a yeast which has been studied under another name (*S. pastorianus, ellipsoideus*, etc.). These, in their scums, present often the appearance described by Reess in *S. conglomeratus*. The existence of this yeast is, then, problematical.

SACCHAROMYCES HANSENII. Zopf [2]

This yeast has been isolated from the pollen of cotton. The cells are spherical or ellipsoidal and each one contains many fat globules. They measure 4 to 11 μ. The ascospores are spherical and to the number of two per asc. The cultures on must gelatin in stabs are a brilliant white with no liquefaction. In solution of dextrose, d-galactose, lactose, maltose, dulcite, glycerol and mannite, this yeast produces varying amounts of oxalic acid.

SACCHAROMYCES THEOBROMAE. Preyer [3]

This yeast completes the fermentation of cocoa. In scums, its cells are long, cylindrical rods. In culture solutions when poorly

[1] Reess, M. Ueber den Soorpliz Sizungsber. der physikalisch. med. Sozietät. Erlangen Botan. Ztg. 1878.

[2] Zopf, W. Oxalsäuregärung bei einem typischen Saccharomyceten (S. Hansenii). Ber. Bot. Gesell. 7, 1889.

[3] Preyer, A. Der Tropenflanzer, 1901.

nourished, this species produces ascospores after 18 hours at 25° C. They are very numerous in each asc. In decoctions of cocoa this yeast produces an alcoholic fermentation and forms a scum. It does not invert saccharose and dies rapidly in this solution.

YEAST FROM SALT. Höye [1]

This species was found by Höye in the analysis of air. This author used as a nutrient medium a wheat paste to which was added 17 per cent of salt. It is a round yeast which forms a single spore in each asc. The best medium is a fish bouillon with 10 per cent of salt added. In nutrient liquids with 3 per cent of salt, development ceases completely. The different proportions of salt have no influence on the shape of the cells. This yeast produces no fermentation in apple juice.

SACCHAROMYCES ANGINAE. Achalme and Troisier [2]

This species was found by Achalme and Troisier in a clinical angina analogous to thrush. The cells are oval (8 to 9 by 5–6 μ), isolated in groups of 8 to 10, often budding at one of the poles. (Fig. 123.) In cultures, they produce ascs with 4 rounded ascospores (2 microns). On gelatin, this yeast develops with grayish white colonies, with the deep colonies brown and spherical. *S. anginae* does not liquefy the gelatin. On agar the colonies are thick and of a dull rose. In acidulated water, the growth is cloudy and there is produced at the end of three days a sticky brown deposit. This yeast ferments saccharose.

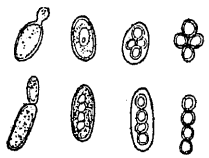


Fig. 123. — *Saccharomyces anginae*. Budding Cells and Ascs (after Troisier and Achalme).

SACCHAROMYCES TUMEFACIENS. (Curtis) Busse

Syn.: SACCHAROMYCES SUBCUTANEOUS TUMEFACIENS. Curtis [3]

This species was observed by Curtis from a tumor of the hip and from a lumbar abscess in man. In the tumor it presents oval or spherical cells with granular contents (16 to 20 μ) and is surrounded by a gelatinous capsule (Fig. 124.) In cultures, the cells oval at first

[1] Höye, K. Rech. sur la moisissure de Bacalao et quelques autres microorganismes halophiles. Bergens Museums Aarbog. No. 12, 1906.

[2] Achalme and Troisier. Sur une angine parasitaire. Arch. med. Exp. 3, 1895.

[3] Curtis, F. Contribution à l'étude de la Saccharomycose humaine. Ann. Past. Inst. 10, 1895.

without capsules sometimes form in chains of two or three. The capsule may develop in old cultures. This yeast often forms asci with from 1 to 4 ascospores (Busse). In gelatin stabs, a white growth is secured after 48 hours. The gelatin is not liquefied. On agar the colonies are small, and lunctiform and fuse quickly into a solid mass. On glycerol potato the growth is rapid. The culture has the appearance of a continuous dry streak, at first white and later brown. On liquid media, beer wort, the development is rapid and abundant. No scum is formed. This yeast inverts saccharose, and causes a feeble fermentation. Alcohol and acid are formed. There is no action on maltose nor lactose. Its maximum temperature is around 37° C. This yeast has a local pathenogenicity for the rat, the white mouse, and the dog.

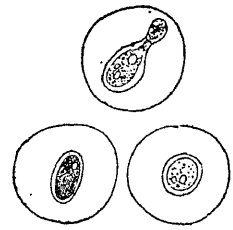


Fig. 124. — *Saccharomyces tumefaciens.* Parasitic Forms Supplied with Gelatinous Capsules.

SACCHAROMYCES GRANULATUS. Vuillemin and Legrain

This yeast was isolated by Vuillemin and Legrain.[1] It possesses oval or elliptical cells, sometimes globular or elongated, about 2–10 $\mu \times 2$–4 μ. The membrane is covered with granulations which are either irregular or regular. (Fig. 125.) These cells form one, rarely two or three buds, and contain fat globules of a reddish color. The cultures thus take on a vermillion tint. Some of the cells are able to encyst and change into durable cells or chlamydospores. On plaster blocks, the cells have very thin and folded membranes containing two or four ascospores which are spherical or elliptical. On liquid media this yeast does not produce a scum but a sediment is formed which is reddish in color. In gelatin stabs, punctiform colonies are formed. The gelatin is not liquefied. On agar, beet, carrot, or cabbage, it forms a folded and shiny coating. On potato slants, the growth is dry. This yeast is pathenogenic for the rabbit.

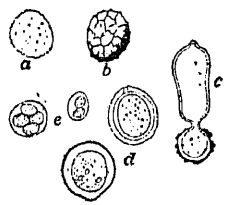


Fig. 125. — *Saccharomyces granulatus.*
a, *b*, and *c*, Vegetative Cells; *d*, *Chlamydospores*; *e*, Asci.

SACCHAROMYCES BLANCHARDII. (Blanchard). Guiart [2]

This yeast described by Blanchard,[3] Schwartz and Binot was isolated from a tumor of a man. It was taken from the peritoneum after

[1] Vuillemin, P., and Legrain. Sur un cas de Saccharomycose humaine. Arch. de Parasit., 3, 1900.

[2] Guiart, J. Précis de parasitologie. Baillière, 1910.

[3] Blanchard, R., Schwartz, E., and Binot J. Sur une Blastomycose intrapéritonéale. Arch. de Parasit., 7, 1903.

which the patient succumbed to the operation. It has round cells, (1.5 to 15 or 20 μ) slightly greenish, surrounded by a thick mucilaginous capsule. In cultures on carbohydrate agar, the cells are also spherical and often appear in beaded formation. (Fig. 126.) Almost all of them after a few weeks transform into spherical asces with a thick wall. They contain 8 round ascospores. These measure 34 μ in diameter. This species grows on liquid media and produces a sediment. On gelatin streaks, it gives a grayish white colony. The gelatin is slowly liquefied. On gelatin plates, white colonies are produced which are round or scalloped and on agar a thick growth, a little yellow and not scalloped. On agar plates, it forms lenticular spots yellow or grayish. On potato, a mucous coating, white or yellowish, is formed and on carrot an abundant viscous growth.

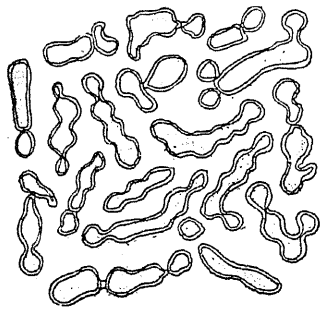


Fig. 126. — *Saccharomyces Blanchardi.* Sucrose Agar Culture after 48 Hours (after Blanchard, Schwartz, and Binot).

SACCHAROMYCES MINOR. Engel[1]

The yeast was found in a fermentation of bread. It has spherical cells 6 μ in diameter, united in chain formation and in little groups of six or nine cells. The asces are 7.8 μ and contain 2 to 4 ascospores of 3 μ in diameter.

SACCHAROMYCES UVARUM. Beijerinck[2]

This yeast is not well known. It was found in a bottle of grape juice to which saccharose had been added. It produced an active fermentation and was associated with *S. sphaericus* (Naegeli). It is a yeast which especially ferments maltose. In yeast water to which maltose is added it produces acetic acid. On nutrient gelatin, asces are formed easily and in large numbers with 4 ascospores.

[1] Engel, Les ferments alcooliques. Thesis for the Doctorate of Sciences, Paris, 1872.

[2] Beijerinck, M. W. Ueber Regeneration der Sporenbildung bei Alkoholhefen. Cent. Bakt. 4, 1898.

SACCHAROMYCES LEMONNIERI. Sartory and Lasseur [1]

Isolated from the sputum of a person with bronchitis and pulmonary congestion, this yeast has round cells surrounded with a thick capsule. It grows easily on most media. The optimum temperature for growth is between 25 and 30° C. It vegetates quite well at 37.5° C. but stops at 40° C. It forms a scum on glycerol bouillon. The temperature limits for scum formation are, 15–20° C. and 37–38° C. Asc formation has been obtained on plaster blocks, each asc containing four spores which are spherical in shape (2.5–3 μ). On carrot, development is rapid, a white thick colony being obtained. On potato, the growth is scant. It is pathogenic for rabbits and guinea pigs.

Genus X. Hansenia. Lindner-Klöcker

HANSENIASPORA. Zikes

Cells lemon shaped, with hemispherical ascospores, and provided with a more or less projecting collar which gives them the appearance of a hat. They germinate by ordinary budding.

HANSENIA APICULATA. Lindner [2]

Syn.: HANSENIOSPORA APICULATA. Zikes

Reess has designated under the name *Saccharomyces apiculatus* a yeast of peculiar shape characterized by the presence at one or both ends of an oval cell, of a little point (nipple) rather long which causes the cell to have an apiculate shape. Hansen [3] has found a yeast which seems to correspond with that of Reess' to which he gave the same name. This species is found in abundance on sugary fruits, in the mucous secretions of trees, the nectar of flowers, etc. It is encountered in the first phases of the wine fermentation.

The cells are apiculate and generally have apiculate buds. Oval cells with oval buds may also be obtained (Fig. 6). Under certain

[1] Sartory, A., and Lasseur, P. New pathogenic yeast (Saccharomyces lemonnieri. Comp. Rend. Soc. Biol. 78 (1915), 48–49.

[2] Lindner, P. Sporenbildung bei S. apiculatus. Woch. f. Brau. No. 42, 1903; Mikroskopische Betriebskontrolle in den Gärungswerben, Paul Parey, Berlin, 6th edition, 1909.

[3] Hansen, E. C. Recherches sur la physiologie et la morphologie des ferments alcooliques. Sur le S. apiculatus et sa circulation dans la nature. Comp. Rend. du labor, de Carlsberg, Vol. 1, Book 4, 1881.

circumstances the cells may be half-moon shaped or elongated. The buds, in the form of a lemon, develop especially in the first phases of the culture and later may be replaced by buds oval in shape. The cells never contain glycogen.

Engel has reported the observation in this yeast of ascogenous fructification related to that of *Protomyces* and has created the genus *Carpozyma* for it. On the contrary, Hansen has never noticed ascs or other forms of fructification in this yeast and regards it as belonging to the *Torula*. However, Beijerinck (1894) reported the presence of ascs with many ascospores. Klöcker, however, has been unable to confirm the presence of ascs and thinks that Beijerinck has taken for ascospores the fat globules which are commonly present in the cells of this yeast.

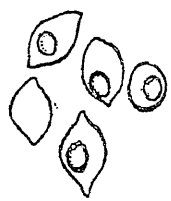


Fig. 127. — Ascs in *Hansenia apiculata* (after Lindner).

Lindner has demonstrated the formation of ascs in a *Saccharomyces apiculatus* isolated from the flowers of *Robinia pseudoacacia*. The ascs never contain but a single ascospore. (Fig. 127.) He was not successful in observing the germination of these ascospores. Röhling has been able to follow the germination of one of these ascospores in a decoction of horse manure to which 5 per cent of glucose was added. It is then probable that special conditions are necessary for their germination.

In the light of these discoveries, Lindner has created for this species a new genus *Hansenia*. But according to Klöcker,[1] *Saccharomyces apiculatus* in which Lindner has described ascospores, may not be identified with the *Saccharomyces apiculatus* of Hansen but may only be a species related to this yeast, for in the true *S. apiculatus*, under no circumstances may the presence of ascs be observed. Indeed, all of the efforts put forth by Hansen and Klöcker to demonstrate spores in this species have been in vain.

Zikes,[2] on the other hand, has tried to make *Saccharomyces apiculatus* sporulate by various procedures but has had little success. He admits that *Hansenia apiculata* is different from *S. apiculatus* and proposed to designate the *Saccharomyces apiculatus* of Hansen under the name of *Hanseniaspora mucroniata* (Lindner). The genus *Hanseniaspora* would be part of the family of Saccharomycetes while the genus *Hansenia* would be placed among the *Non-Saccharomycetes*. It may be regarded as an asporogenic variety of *Hansenia* or as a special form not having all of the characteristics of the latter.

[1] Klöcker, A. Invertin und Sporenbildung bei Saccharomyceten apiculatus-Formen. Cent. Bakt. 21, 1910.

[2] Zikes, H. Zur Nomenclaturfrage der Apiculatushefe. Cent. Bakt. 30, 1911.

We owe to Klöcker [1] a very important study of apiculate yeasts. This author has isolated a series of forms made up of different species which have been described under the name of *Saccharomyces apiculatus*. Klöcker concluded that this is not a special species but simply a group of species. He has isolated two groups of species which do not sporulate and which he has incorporated in the family of *Torulaceae* or *Non-Saccharomycetes* with the generic name of *Pseudosaccharomyces*, replacing the genus of *Hansenia* of Zikes. The sporulating species he has placed in the *Saccharomycetes* under the name of *Hansenia* (Lindner), replacing the genus *Hanseniaspora* of Zikes.

HANSENIA VALBYENSIS. Klöcker

On beer wort, at 27° C., the cells are apiculate or shaped like *ellipsoideus;* some are shaped like short sausages (5–8 μ). The limits of temperature for growth are 32°–33° C. and 0.5° C. The spores appear at the end of from 4 to 5 days on wort gelatin at 25° C. They are hemispherical and to the number of two per asc, rarely one. It ferments dextrose, levulose and d-mannose. Gelatin is liquefied. It was found at Copenhagen.

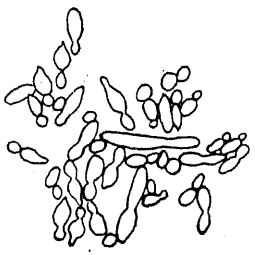


Fig. 128. — *Hansenia Valbyensis.* Cells after 3 Days on Wort at 30° C. (after Klöcker).

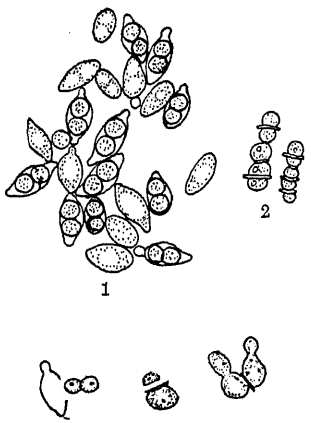


Fig. 129. — *Hansenia valbyensis.* 1, Asci; 2, Ascospores; 3, Germination of Ascospores according to Klöcker.

FOURTH GROUP

Budding yeasts, without copulation in the origin of the asc. These yeasts form a scum on sugar media which is dry and opaque. The ascospores are hemispherical in the form of a lemon supplied with a projecting collar and a single thick membrane. Germination is sometimes preceded by a parthenogamy. The greater part of the species in this group do not give an alcoholic fermentation but do produce ether.

[1] Klöcker, A. Recherches sur les organismens de la fermentation. II. Recherches sur 17 formes des Saccharomyces apiculatus. Comp. Rend. Trav. lab. Carlsberg, 1913.

Genus XI

Spherical or hemispherical ascospores, irregular or angulous in form. Generally no fermentation is produced. Forms a strong mycelium.

PICHIA MEMBRANAEFACIENS. Hansen

Syn.: SACCHAROMYCES MEMBRANAEFACIENS. Hansen

This species was found by Hansen in the gummy exudations of the elm, by Koehler in well water and by Jörgensen in white wine. It has been described by Hansen [1] and Siefert.[2] It resembles the Mycodermae (*Mycoderma cerevisiae*, or *vini*). When cultivated on beer wort a thick scum is rapidly formed, folded and grayish in color. It is filled with air bubbles. The cells are spherical or elongated and rich in vacuoles (Fig. 130). The temperature limits for budding in beer wort are: minimum, 0.5° C. and maximum, 35° C.–36° C. The scum is not formed at the temperature limits and the yeast vegetates, then, as a deposit.

The asci form easily on plaster blocks and also in most of the culture media and especially in the scums. They possess two spherical, elongated, hemispherical or oval ascospores, sometimes triangular, which measure about 4.5 microns in length. According to Nielsen,[3] the maximum temperature for budding is situated between 33 and 35° C., the minimum temperature between 2.5 and 2.7° C. and the optimum between 30.5 and 31° C. At this latter temperature the ascospores are formed in 19–21 hours. On wort gelatin, the colonies develop on the surface of the substratum and look like a shield. They are rugose and have a reddish tint. The gelatin is liquefied very rapidly. The colonies which develop in the depths of the medium have a different appearance and liquefy gelatin less rapidly. This species does not invert saccharose, and according to Hansen, does not ferment any sugar. However, Lindner has caused a slight fermentation of dextrose and levulose.

[1] Hansen, E. C. Recherches sur la physiologie et la morphologie des ferments alcooliques. VII, Action des ferments alcooliques sur les diverses espèces de sucre. Levures alcooliques à cellules ressemblant à des Saccharomyces. Comp. Rend. du. lab. de Carlsberg, 2, Book 5, 1888; XI, La spore de Saccharomyces devenue sporage. Recherches comparatives sur les conditions de croissance vegetative et le développement des organes de reproduction des levures et des moisissures. Comp. Rend. du Lab. de Carlsberg, 5, Book 2, 1902.

[2] Siefert, W. Saccharomyces membranaefaciens. Ber. d. chem. Phys. Versuchsst. Jlosternburg, 1899–1900.

[3] Nielsen, J. C. Sur le développement des spores du *Saccharomyces membranaefaciens*, du *Sacch. Ludwigii*, et du *Sacch. anomolous*. Comp. Rend. du lab. de Carlsberg, 3, Book, 31, 1891.

PICHIA MEMBRANAEFACIENS II AND III. Pichi

Syn.: SACCHAROMYCES MEMBRANAEFACIENS II AND III. Pichi

Pichi [1] has described two species of yeast which very much resemble *P. membranaefaciens* and which he has called *Saccharomyces membranaefaciens II* and *Saccharomyces membranaefaciens III*. These two species have somewhat the same physiological and morphological characteristics of *Saccharomyces membranaefaciens* of Hansen. They seem to differ only in the shape of their ascs.

P. membranaefaciens II was found on the leaves of *Evonymus europaeus*. The cells are usually 5 to 7 μ long and 3.5 μ wide and even 10–19 μ long and 3–4 to 5 μ wide. The ascospores are round or a little flattened (2.5–3 μ). The ascs enclose 3–4 ascospores and form in a milky white, folded scum. They are oval (6–8 μ long by 3–5 μ wide).

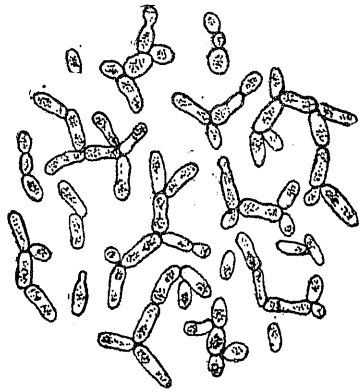


Fig. 130. — *Pichia membranaefaciens* (after Kayser).

P. membranaefaciens III was isolated from wine. The cells are 5 to 7 μ long and 3–4 to 6 μ wide. The ascospores (2.5 × 3.5 μ) are to the number of 2–4 in a spherical or oval asc. The scum develops on beer wort at 22–25° C. and is folded with a large number of ascs.

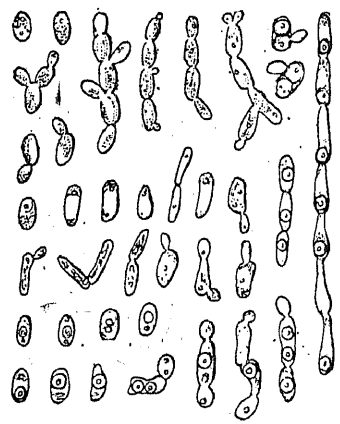


Fig. 131. — *S. hyalospora.* Culture on Saccharose Gelatin. Vegetative Cells and Ascs (after Lindner).

PICHIA HYALOSPORA. Lindner

Syn.: SACCHAROMYCES HYALOSPORUS. Lindner

This yeast, discovered by Lindner, forms on beer wort a delicate scum. The ascospores are rounded, each being provided with a brilliant granule in the center probably of the nature of a fat globule (Fig. 131). The giant colonies are a dull gray with a filaceous appearance. This yeast does not induce a fermentation.

[1] Pichi, P. Ricierche morph. e fisilogische sopra due nuove specie di Sacch. prossime al S. membranaefacfens. Ann. della R. Scuola di viticoltura e di Enologia in Conegliano, 1, 1892.

PICHIA TAURICA. Siefert

Syn. SACCHAROMYCES MEMBRANAEFACIENS, *var.* TAURICUS. Siefert

This species was found by Siefert [1] in Crimean wine. It forms a delicate scum which falls to the bottom of the culture flask very easily. This scum is composed of elongated or sausage-shaped cells (20 μ long and 4–6 μ wide) rarely oval. The temperature limits for budding are: maximum, 22° C., minimum, 5–6° C. on wine with 8 per cent of alcohol. The optimum is about 22° C. Numerous ascs are formed after a short time in the scum. The temperature limits for sporulation are 5–6° C. and 34° C. This yeast does not grow in more than 12.2 per cent of alcohol by volume.

PICHIA TAMARINDORUM. Siefert [2]

Syn: SACCHAROMYCES MEMBRANAEFACIENS *var.* TAMARINDORUM. Siefert

Isolated from an alcoholic drink prepared with tamarind, this yeast possesses elongated cells rarely oval or pear shaped, with a very refractive granule in the protoplasm. The elongated cells are about 2–6 μ in length and 2–6 μ in width. The oval cells are about 5–6 μ in length and 2–3 μ in width.

The scum is thick powdery white, and wrinkled in old cultures. If it is disturbed, it falls to the bottom of the flask in large flakes. Spores are abundantly produced in the scum at temperatures of the laboratory. They are also produced easily on plaster blocks between 27 and 30° C. At 34° C. and at 1.5° C. no ascospores are formed. The ascospores are hemispherical (3 to 4 μ) and usually possess a refractive granule in the center. On gelatin the giant colonies have a characteristic rosette appearance.

PICHIA CALIFORNICA. Siefert

Syn.: SACCHAROMYCES MEMBRANAEFACIENS *var.* CALIFORNICUS. Siefert [1]

Discovered by Siefert in red wine from California, this yeast generally possesses oval cells (4–8 μ in length and 3–5 μ in width) enclosing a refractive body. It forms a delicate scum which falls to

[1] Siefert, W. Ueber die Säuerabnahme im Wein und den dabei stattfindenden Gärungsprozess. Zeit. landw. Versuchswesen in Osterreich. 1900.

[2] Siefert, W. Saccharomyces membranaefaciens. Ber. d. Chem. physiol. Versuchsst. Klosterneuburg. 6, 1899–1900.

the bottom of the flask when disturbed. The temperature limits for budding are 7–12° C. and 33° C. in wine with 8 per cent of alcohol. The optimum is situated between 28° and 30° C. In beer wort the maximum temperature is near 39° C. The temperature limits for sporulation are 5–6° C. and 39–40° C. The optimum is near 34° C. The ascospores are spherical and refractive (2–3 μ in diameter) and develop only in 12.2 per cent of alcohol by volume. This species forms less glycerol in Pasteur's medium and does not attack alcohol as much as *P. membranaefaciens*. *Pichia californica* has also been found by Saito [1] in the fermentation of rum from molasses on the island of Bonin, Japan.

PICHIA RADAISII. Lutz

Syn.: SACCHAROMYCES RADAISII. Lutz [2]

This species was isolated by Lutz from tivy, an alcoholic drink, prepared in Mexico. It has oval or elongated cells (8 to 8.5 μ long and 3 to 3.5 μ wide). The optimum temperature for the formation of a scum is 23° C. Budding ceases at about 37–38° C. Sporulation is accomplished at the end of 12 hours at 22°–23° C. The maximum temperature for the formation of ascospores is between 25 and 28° C. The ascospores (1.4 μ in diameter) are spherical and generally to the number of 4 per asc. This species does not liquefy gelatin. The colonies are reddish in color which appear after a culture period of some time.

PICHIA FARINOSA. Lindner

Syn.: SACCHAROMYCES FARINOSUS. Lindner

This species was found by Lindner [3] in beer in Danzig. Saito has recently found it among the yeasts in soy bean sauce. The cells are generally elongated; some are ellipsoidal (Fig. 127). The maximum temperature for the formation of the scum is 37° C. On beer wort, or decoction of "koji," it forms a dry scum after 24 hours. Ascospores are formed easily in most media notably in the scum, but the property of forming spores is completely lost by cultivating on gelatin. The ascospores are to the number of 1 to 4 per asc. They are rounded or oval with a brilliant granule in the center. According to the illustrations of Lindner and Saito it seems that the ascs are derived

[1] Saito, K. Notiz über die Melasse-rumgärung auf den Bonin-inseln. (Japan). Cent. Bakt. 16, 1908.

[2] Lutz, C. Rech. sur la constitution du Tiby. Bull. Sic. myc. de France, 15, 1899.

[3] Lindner, P. Saccharomyces farinosus and Bailii. Wochensch. f. Brau. 1893.

from a copulation. These authors show asci formed from two cells united by a canal. Sometimes only one will produce ascospores and sometimes both. (Fig. 132.) Guilliermond [1] has shown that these illustrations simply represent budding. In fact, the cells destined to sporulate are able to continue budding at the moment of sporulation and the ascospores often form before the separation of the cell which is formed in the budding process. Sometimes the bud will form on the lateral surface of the cell, then will elongate parallel with the mother cell which results in forms resembling the *Zygosaccharomyces*.

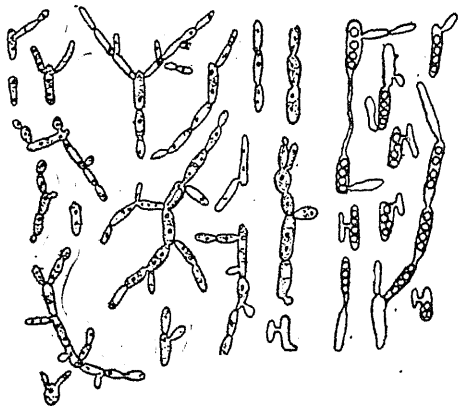


Fig. 132. — *Pichia farinosa*. Vegetative Cells and Asci in Scum on Beer Wort (after Lindner).

The giant colonies on maltose gelatin are circular and have a chalky white appearance. On gelatin streaks, the culture is also whitish and the edge finely indented. Liquefaction of the gelatin is quite rapidly accomplished. The giant colonies are chalky white with a folded surface and slowly liquefy the gelatin. This yeast feebly ferments dextrose and levulose but has no action on other sugars (maltose, d-galactose, saccharose, melibiose, raffinose, α-methylglucosides). In beer wort, the amount of alcohol produced is very small. In 11 days, 0.9 per cent of alcohol and ethyl ether is formed. According to Siefert, *Pichia farinosa* produces in alcoholic must acetic acid.

PICHIA SUAVEBLEUS. Klöcker

Found in soil in Denmark, this species forms on must at the end of 24 hours at 25° C. a gray scum which adheres to the walls of the flask, and a white thick ring. On beer wort the scum is thick and compact with a yellowish color. The cells in the scum are spherical or oval (5 to 8 μ long). The temperature limits for the formation of scum are 34–36° C. and 10–4° C. The ascospores are spherical sometimes flattened on one side with



Fig. 132–bis. — *Pichia suavebleus*. Cells from a Young Scum on Beer Wort (after Klöcker).

[1] Guilliermond, A. Quelques remarques sur la sexualite des levures. Annals. mycologici, 8, 1910.

a large refractive globule in the center. Each asc contains about 2 ascospores. Ascospores are formed with difficulty on plates. The temperature limits for sporulation on plates are 29–33° C. and 10° C. It ferments dextrose and saccharose only. In beer wort it sets up a feeble fermentation with a fruity odor. Gelatin is not liquefied.

PICHIA ALCOHOLOPHILA. Klöcker

This species was found in Denmark. On must after a few days it forms a scum at 25° C. more or less developed. On must with 10 per cent of alcohol added, it forms a well-developed scum. It is folded and adheres to the walls of the flask. There is abundant sedimental growth. The cells in the scum are spherical; those of the sediment are oval or sausage shaped. The temperature limits for the formation of the scum are 33–35° C. and 3.4 to 8.4° C. The ascospores are to the number of four, sometimes two per asc. They are spherical or hemispherical. They are formed abundantly in the scums. The limits of temperature for the formation of spores on plates are 29–33° C. and 0.5–4° C. Gelatin is partially liquefied. Dextrose is feebly fermented.

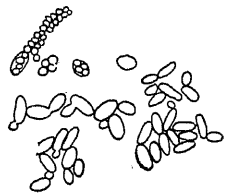


Fig. 132–A. — *Pichia Alcoholophila* (after Klöcker). Cells from a Young Scum on Beer Wort to which Alcohol was Added.

PICHIA POLYMORPHA. Klöcker



Fig. 132–B. — *Pichia polymorpha* (after Klöcker). Cells from a Young Scum on Beer Wort at 25° C.

This species was also found in the soil of Denmark. A white well-developed scum is formed on must in 24 hours at 25° C. It adheres to the sides of the flask. At first the cells are elongated or egg-shaped (13 μ long). They bud laterally. Finally, they become spherical or oval. The temperature limits for scum formation are 39° and 0.5° C. The ascospores are spherical and formed with difficulty on plates. Gelatin is liquefied. Dextrose and saccharose are fermented and maltose feebly.

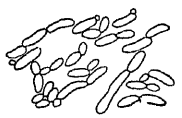


Fig. 132–C. — *Pichia calliphora* (after Klöcker). Cells from a Young Scum on Beer Wort at 25° C.

PICHIA CALLIPHORA. Klöcker

This species was found on the fly *Calliphora arthrocephala* at Carlsberg. On beer wort, a white scum is formed along with a feeble ring. The cells are sausage shaped and almost 13 μ long. The temperature limits for scum formation are

33–35° C. and 0.5–4° C. The ascospores are small, spherical or hemispherical to the number of 2 to 4 per asc. The temperature limits of sporulation on plates are 24–27° and 7–10° C. Gelatin is liquefied. A fermentation is set up in wort. Dextrose only is fermented.

PICHIA MANDSHURICUS. Saito

This was isolated from Chinese yeast used in the preparation of an alcoholic drink in Manchuria. It was found along with *Zygosaccharomyces mandshuricus*. The temperature limits for scum formation are 7–10° C. and 40–41° C. The spores are globular or spherical (2–4 μ) obtained in the scums on plaster blocks, 1 to 4 per asc. The temperature limits for sporulation are 11–16–32° C. Dextrose is fermented.

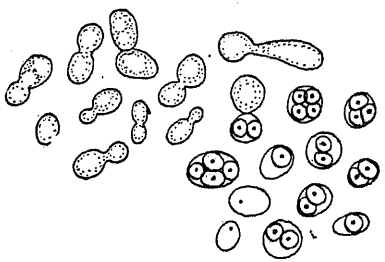


Fig. 132–D. — *Pichia mandshuricus* (after Saito).

At the same time Saito isolated another species of the same group named *Kocoling chiu Kahmhefe* which has elongated cells. The temperature limits for the formation of the scum are 7–10° C. and 60° C. It ferments dextrose, fructose and mannose. Only once on Gorodkowa's gelatin did the author observe spore formation.

YEAST FROM PULQUE NO. 1. Guilliermond[1]

This species was isolated from the fermentation of Pulque. It belongs to the genus *Pichia*. On beer wort, at 25–30° C., there are formed at the end of a day, small floating patches on the surface of the liquid and after 48 hours, a scum which is grayish white in color. A sedimental growth is also formed. The cells are generally oval, sometimes round. In old cultures, they are elongated and remain in chain formations. The maximum temperature for growth is near 30° C., the optimum being between 29° C. and 30° C. The ascospores appear on most media. They are to the number of 1 to 4 per asc, and have a hemispherical shape. The maximum temperature for sporulation is around 38–39° C. The optimum is between 29–32° C. The yeast inverts saccharose but produces no fermentation.



Fig. 132–E. — Yeast from Pulque No. 1. Cells from Carrot (after Guilliermond).

[1] Guilliermond, A. Levaduras del Pulque. Boletin de la Direccion de Etudios Biologicos, Mexico, 2, 1917.

PICHIA ORIENTALIA. Beijerinck [1]

This yeast is little known but seems to be related to the genus *Pichia*. It possesses ascs which makes it improper to class it with the *Pichia*. It was isolated by Beijerinck from a sample of "koji" secured from Eyckman. The same author has found it on certain oriental fruits (grapes from Corinth). It is a yeast which especially ferments dextrose. In yeast water to which glucose has been added, it brings about an active fermentation. In beer wort the fermentation is less active. It does not ferment maltose. On beer wort it forms a scum and produces carbon dioxide. In culturing it on grape must to which lactic acid has been added, at 28° C., it produces a dry scum, powdery, in which numerous ascospores are formed.

SACCHAROMYCES MYCODERMA PUNCTISPORUS. Melard [2]

This yeast which seems to belong to the *Pichia* was isolated by Melard in a Belgian brewery from a top fermentation. It causes a disagreeable taste in beer. The cells belong to the *Pastorianus* or *ellipsoideus* type and show, in their interior, from 1 to 3 small black points. The yeast is essentially aerobic and develops a scum rapidly on liquid media. This scum varies with the medium. It may present a scum like ordinary yeasts or be folded and present characteristics of the scum of *Mycoderma*. Sporulation has been obtained on plaster blocks and in scums. At the temperature of 15–18° C. it is accomplished in 5 to 6 days. The ascospores may result from an increase in size of the black spots in each cell. The giant colony is dull and of a yellowish tint. It does not liquefy gelatin. This species does not produce any liquefaction. Levulose is preferred over other sugars as a food. It does not invert saccharose.

Genus XII. Willia. Hansen

Ascospores in the shape of a lemon or hat with a projecting ring around them. Most of the species produce ethers but some give only an alcoholic fermentation.

[1] Beijerinck, W. Ueber regeneration der Sporenbildung bei Alkoholhefen wo diese Funktion in Verschwinden begriffen ist. Cent. Bakt. 4, 1898.

[2] Melard, L. Note sur un organisme isole d'une bière de fermentation haute. First International Congress of Brewing, Brussels, 1910

WILLIA ANOMALA. Hansen

Syn.: SACCHAROMYCES ANOMALOUS. Hansen

This species was isolated by Hansen [1] from an impure brewery yeast which came from Bavaria. The cells of this yeast resemble those of *Torula* discovered by Hansen. They are small cells, generally oval, often sausage shaped. In the beginning of fermentation, this yeast forms a dull gray scum which resembles very much that of *Monilia candida*. Among the cells in the scum are found many air bubbles. The temperature limits for budding on beer wort are 0.5–1° C. and 37–38° C. At these limits of temperature no scum is formed. The yeast, then, develops as a sediment.

Ascospores appear very easily at the end of a short time, as well in the scum as in the cells in the deposit. They are formed easily in most solid media in most favorable conditions of nutrition. The temperature limits for sporulation on plaster blocks are, according to Nielsen, 32–34° C. and 2.5–7.5° C. The optimum is at 30° C. At this temperature the ascospores begin to form in 17–18 hours.

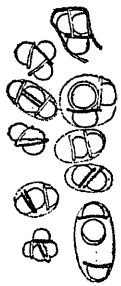


Fig. 133. — Asci of *Willia anomala* (after Hansen).

The number of ascospores varies from 2 to 4 in each asc. They may be located in the asc in a diversified manner. Their diameter is about 2 to 3 μ. They possess a characteristic shape absolutely analogous to those of *Endomyces decipiens*, *Endomyces fibuliger* and *Ascoidea rubescens*. They are hemispherical and shaped like a hat. (Fig. 133.) The wall of the asc is broken easily. Germination of the ascospores is brought about in the following manner. The ascospore swells up during which the projecting collar may persist or disappear completely. The ascospore produces, at different points on its surface, buds which multiply in their turn by budding. (Fig. 38.) This yeast ferments beer wort rapidly. During the fermentation the liquid is stirred up becoming opalescent, and gives off a fruity odor. This species ferments dextrose but not saccharose or maltose. *Willia anomala* has been found by Klöcker and Schiönning, Kozai and Saito in "koji" employed in the preparation of awamori, an alcoholic drink on the island of Luchu.

BIOLOGICAL VARIETIES OF WILLIA ANOMALA

Since the discovery of this species, numerous varieties of the type *anomalous* have been observed all of which have special characteris-

[1] Hansen, E. C. Recherches sur la physiologie et morphologie des alcooliques ferments. VIII. Sur la germination des spores chez les saccharomyces. Comp. Rend. du lab. de Carlsberg 3, 1891; 5, 1902.

tics, such as the special shape of their cells or the odor which they give off in sugar solutions. Zeidler has described it in the juice of the marsh mallow and Jörgensen in an English yeast. Beijerinck has described under the name of *Saccharomyces acetaethylicus* a species producing ethyl ether which seems to be a member of the *Willia anomala* group. The same author isolated a variety of *Willia anomala* which he called *Mycoderma pulverulenta;* these two species have been insufficiently described. Finally, Fischer and Brebeck have encountered another variety under the name of *Endoblastoderma* which forms, like the *Willia anomala* of Hansen, a white powdery scum, but differs in the method of formation of endogenous germ. In certain cells it forms a sort of internal spore which is placed well against the wall of the cell. This remains attached to the cell like a bud after it has been released from it. Klöcker has shown that these endogenous formations are due to an optical illusion caused by budding and that *Endoblastoderma* of Fischer and Brebeck does not differ from *Willia anomala* but may be identified with it. Other authors (Holm, Meissner, Will, Lindner) have found forms related to *Willia anomala.* Beauverie and Lesieure have isolated one from sputum in pulmonary tuberculosis. Finally, Steuber [1] has described a series of biological varieties of *W. anomala* which we shall describe briefly.

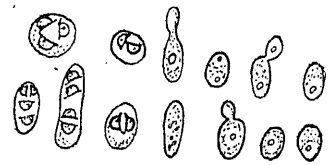


Fig. 134. — *Willia anomala* of Zeidler on Wort Gelatin (after Lindner).

WILLIA ANOMALA I. Steuber

Syn.: SACCHAROMYCES ANOMALUS I. Steuber

This variety is characterized by the aromatic odor produced by ethyl ether in its cultures. On beer wort, it forms a scum at first folded and yellow. The temperature limits for the formation of a scum are 5–10° C. and 37°–42° C. The optimum is 30° C. Sporulation is accomplished on plaster blocks and rarely in scums or giant colonies. The temperature limits for sporulation on plaster blocks are 5–12° C. and 30°–35° C. Ascospores appear at the end of 13 to 14 hours at the optimum temperature on plaster blocks. They rarely form in scums and giant colonies. The ascospores have the form of a hat. The giant colony is yellow in the center and white or shiny at the edge. Gelatin is liquefied. This variety ferments 10 per cent solutions of saccharose, dextrose and levulose. It does not act on maltose, lactose or d-galactose which it uses in its nutrition. In liquids

[1] Steuber, L. Beiträge zur Kentniss des Gruppe Saccharomyces anomalus. Zeitsch. d. ges. Brau. 23, 1900.

containing sugars which it ferments, it forms ethyl ether and acetic acid, sometimes a little butyric acid.

WILLIA ANOMALA II. Steuber

Syn. SACCHAROMYCES ANOMALUS II. Steuber

This variety forms on beer wort a scum which is at first folded and chalky but which later assumes a rose tint. The temperature limits for the formation of a scum are 5 to 10° C. and 30°–35° C. The optimum is about 30° C. The formation of ascospores is easily accomplished on media in 44 hours. The temperature limits for sporulation on plaster blocks are 5°–15° and 30°–35° C. The ascospores are hat shaped. After a certain time the giant colonies are rose colored or reddish brown. It liquefies gelatin. This variety inverts saccharose which it ferments slowly but completely. It produces only traces of alcohol in 10 per cent solutions of levulose. It has no action on other sugars. It does not form ether or fatty acid but traces of acetic and butyric acids.

WILLIA ANOMALA III. Steuber

Syn.: SACCHAROMYCES ANOMALUS III. Steuber

This variety came from brown Munich beer. The scum is white, later yellow. The temperature limits for its formation are 5–15° C. and 30–35° C. The optimum is 30° C. The temperature limits of sporulation on plaster blocks are 5–15° C. and 30–35° C. The ascospores form in great numbers in giant colonies but never in scums. They are hat shaped. The giant colony is white, irregular and liquefies gelatin. This variety gives only traces of alcohol in solutions of 10 per cent levulose and does not ferment any other sugar. It produces neither ethyl ether nor fatty acids but, at the beginning, only traces of acetic acid and butyric acid which are eventually oxidized.

WILLIA ANOMALA IV. Steuber

Syn.: SACCHAROMYCES ANOMALUS IV. Steuber

This variety has the same origin as the preceding variety. The scum is white and later yellow. Its temperature limits are 5–15° C. and 35–41° C. The temperature limits for sporulation are 15–20° C. and 30–35° C. The ascospores are hat-shaped. The sporogenic property is lost on long cultivation while it is preserved in the preceding variety. This is one method for distinguishing between them. The giant colony is at first white and later yellow and folded. It liquefies

gelatin. This variety forms traces of alcohol in solutions of 10 per cent levulose and acts on no other sugar. From the point of view of acids and ether it acts like the preceding yeast.

WILLIA BELGICA. Lindner

Syn.: SACCHAROMYCES ANOMALUS *var.* BELGICUS. Lindner[1]

This yeast, closely related to *Willia anomala*, was discovered by Lindner in Belgian beer. The cells are small with thin walls. This species produces ascospores which look like those of *Willia anomala*. It is distinguished from *Willia anomala* by the fact that it ferments not only dextrose but d-mannose, d-galactose and levulose and that it does not produce an ether odor. It develops on beer wort in the form of a dotted scum.

WILLIA WICHMANNI. Zikes[2]

Isolated from soil near Vienna this yeast has cells 3 to 5 μ ranging from 6 to 40 μ long in scums. It grows on agar from 5° C. to 42° C. The optimum is 22° C. Sporulation appears rapidly at 21° C. on plaster blocks, less actively at 18 to 28° C. The ascospores are hat shaped (2 μ in diameter) and are to the number of 2, 3 or 4 per asc. This yeast grows on peptone gelatin in a white layer. On glucose agar, the colonies look like little droplets, whitish yellow in color. On potato, the yeast gives a grayish viscous growth. On slices of beet, it produces a yellow layer and abundant development with the formation of numerous ascospores. It causes a cloudy beer wort with the formation of a scum in 3 days. This yeast ferments dextrose and levulose but does not attack maltose, d-mannose, d-galactose, lactose, saccharose, raffinose, inuline or dextrine. It forms ethyl ether in fermentation.

WILLIA ANOMALA. Saito

This yeast forms a thick white scum on must, white and adhering a little to the walls of the container. The cells are round or oval and sometimes in chain formation. The giant colonies on gelatin are white and united. The temperature limits for scum formation are: maximum, 35° C. and minimum, 2–3° C. The spores are shaped like a hat with a projecting edge (2.7–3.6 μ in diameter). They are to the number of 4 per asc. They form abundantly on plaster blocks

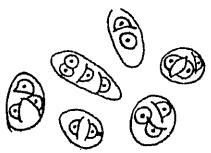


Fig. 134–A. — Ascs of *Willia anomala*, Saito (after Saito).

[1] Lindner, P. Mikroskopische Betriebskontrolle in den Gärungswerben, Paul Parey, Berlin, 6th edition, 1909.

[2] Zikes, H. Ueber anomalus-Hefe und eine neue Art derselben. Cent. Bakt. 16, 1906.

but in scums on beer wort they appear very rarely. The temperature limits for sporulation are 2–3° C. and 30° C. This yeast ferments dextrose, levulose, saccharose, mannose and raffinose.

WILLIA SATURNUS. Klöcker

Syn.: SACCHAROMYCES SATURNUS. Klöcker

This yeast was found by Klöcker[1] in a sample of earth from Himalaya. It has since been found in Italy and Denmark. It develops on must with a white wrinkled growth and a sediment. In a young scum, the cells are globular or oval in shape (4 to 6 μ) (Fig. 135). Later they enlarge, become spherical and filled with globules of fat. At the same time the scum increases, great bubbles of carbon dioxide are formed and the color becomes yellow. If the culture is shaken the scum will fall to the bottom of the culture flask and a ring is formed around the culture flask.

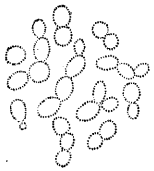


Fig. 135. — *Willia Saturnus*. Cells of Scum on Beer Wort after 24 Hours at 25° C. (after Klöcker).

The temperature limits for budding on beer wort are 2 to 4° C. and 35° to 37° C. The optimum is situated between 28 and 30° C. The scum forms easily on yeast water to which pure saccharose is added. Maltose, dextrose or levulose also serve well.

Sporulation is rapidly accomplished on plaster blocks. The temperature limits are 4 to 7° C. and 28° to 31.5° C. The optimum is near 25° C. At this last temperature, the ascospores appear at the end of 43 hours. They also develop abundantly in the scum on yeast water and rice. They appear both in the ring in the side of the culture tube and in the scums of cultures on must and also in old cultures on gelatin. The shape of the ascospore is that of a lemon but sometimes the points are less evident and indistinct. The ascospore is always girdled with a projecting ring, which reminds one of the planet Saturn, indicating the origin of the name of this yeast. In the interior of the cells, there is a globule probably of fat. The length of the ascospore is about 3 μ and the width 2 μ. The ascs include generally two ascospores, sometimes three, rarely one, and exceptionally four. The germination of the ascospores operates by budding. Often it is preceded by a fusion of ascospores two by two which is accompanied by a nuclear fusion constituting a true copulation (parthenogamy) (Fig. 39).



Fig. 136. — Asci of *W. Saturnus* (after Klöcker).

[1] Klöcker, A. Une espèce nouvelle de la famille Sacchar. (S. saturnus). Comp. Rend. des trav. lab. de Carlsberg, 6, 1903.

On gelatin must, *Willia saturnus* forms white or pale yellow colonies, the surfaces of which are wrinkled or folded. Their form resembles a crater. The cells in the colonies have a spherical form containing numerous drops of fat. Gelatin is liquefied slowly. On a mixed gelatin and peptone bouillon, the cells are small and often a bit elongated. The appearance of the colony is much like that on gelatin must.

Willia saturnus inverts saccharose. It ferments dextrose, raffinose, levulose, but has no action on maltose, lactose or arabinose. In beer wort, it provokes a slow fermentation. It gives off an odor of ethyl ether. Alcohol disappears after a time in cultures, probably being oxidized.

Sartory has found in the juice on banana leaves a yeast which is much like *Willia saturnus* and which may be a variety of it. In beer wort, the cells in the sediment are oval ($7–8 \times 4.5\,\mu$). The optimum temperature for budding on carrot is situated between 32 and 34° C.; the maximum is between 41° C. and 42° C. The yeast forms a scum after 36 hours in glycerol bouillon at 15–18° C., after 2 days at 20–22° C., and after 3 days at 38–39° C. None is formed higher than this. The scum consists of cells like those in the sediment, but when they become old they are spring shaped or like a pseudomycelium. On beer wort gelatin, the yeast forms a dull, white round colony with a banana-like odor.

Sporulation is accomplished on plaster block only on condition that the yeast be associated with the bacteria with which it is found and from which it is separated with great difficulty. The temperature limits for sporulation are a little below 8° and from 22–30° C. The optimum is situated between 15 and 18° C. The asci ($8–9\,\mu$ in diameter) have one to four ascospores ($2–3\,\mu$) similar in shape to those of *Willia saturnus*, which germinate by ordinary budding. The yeast secretes invertase and produces alcoholic fermentation.

Fifth Group

Budding yeasts with uncertain affinities. Fusiform ascospores in the form of a needle.

Genus XIII. Monospora. Metschnikoff [1]

Single ascospore in the form of a needle, germinating laterally in a digitiform prolongation which buds into dissociated oval bodies. This genus is represented by only *Monospora cuspidata*.

[1] Metschnikoff, E. Ueber eine Sprosspilzarankheit der Daphnien. Beitrag zur Lehre über den Krampf der Phagocyten gegen Krankheitserreger. Virchow's Archives, 96, 1884.

MONOSPORA CUSPIDATA. Metschnikoff

This yeast was found by Metschnikoff in 1884, in the general cavity of the *Daphnia*. It possesses cells which are oval and which elongate to form the asc. Each asc includes a single ascospore, very thin and elongated in the form of a needle. It germinates by budding on a side with the formation of oval cells. (Fig. 137.) The ascospores swallowed by the *Daphnia* reach the intestine and finally the general cavity. Here they bud quickly and cause the death of the animal. Metschnikoff, on account of the transparency of this organism, has been able to follow all the steps in the process of phagocytosis taking place with the cells derived from the ascospores.

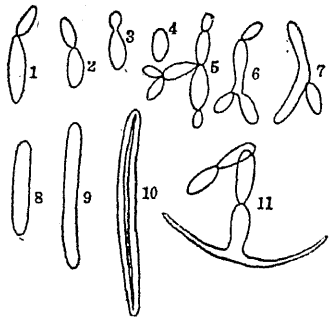


Fig. 137. — *Monospora cuspidata.*
1–7, Vegetative Cells as a Means of Budding. 8–10, Formation of the Asc. 11, Germination of the ascospore (after Metschnikoff).

Genus XIV. Nematospora. Peglion [1]

Ascospores spindle-shaped with a long cilium at one of the extremities. Germination is accomplished by budding at both ends. Many ascospores in each asc. Up to the present only two species are known.

NEMATOSPORA CORYLI. Peglion

This very curious yeast was discovered by Peglion in Italy in 1901 in moldy hazel nut meats. It develops quickly in beef bouillon where it multiplies by budding and produces ascs. The cells are very elongated and possess a double wall (Fig. 138, 8 and 13). In old cultures, they become round or oval. Budding is always accomplished at the poles as in the yeasts of *Dematium*. The ascs develop plain on agar and especially on slices of beet. They are very large (65–70 μ long and 6–8 μ wide). They possess 8 ascospores placed in groups of four in

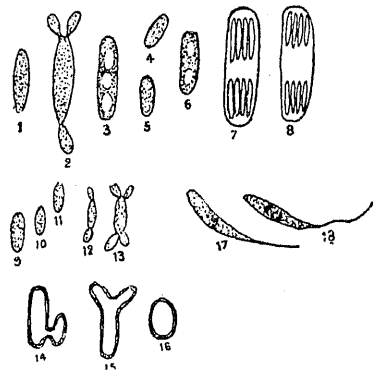


Fig. 138. — *Nematospora coryli.*
1, Ascospore after Disappearance of Cilium. 2 to 6, Germination of Ascospore; 7 to 8, Ascs; 9 to 13, Vegetative Cells in Process of Budding; 14 to 16, Abnormal Forms; 17 to 18, Stained Ascospores with Nucleus and Cilium (after Peglion).

[1] Peglion, V. Ueber die Nematospora Coryli. Notiz, Rendie della Roy. Ac. dei Linei, 1897, Cent. Bakt. 7, 1901.

each half of the cell. (Fig. 138, 7 and 8.) The ascospores are very long: they have the shape of a spindle (2 to 3 μ wide and 38 to 40 μ long) and are equipped with a long cilium at one end. During germination the cilium disappears and the ascospore takes the appearance of a short cell which may produce buds at both extremities. This yeast vegetates only on a solid medium. In liquid media, it stops budding and develops only a sterile mycelium. To this fifth group of yeasts seems to belong a species found by Bütschli in a *Nematode Tylenchus pellucidus.*

NEMATOSPORA LYCOPERSICI. Schneider [1]

Schneider has recently described a yeast isolated from tomatoes secured from a restaurant in Berkeley, California. The proprietor stated that the tomatoes came from the South Sea islands. The tomato appeared to be normal except for the foci of infection which were depressed and reddish brown in color. Schneider characterizes this yeast as follows:

"Asci of gametic origin soon becoming free from associated cells, cylindrical with rounded ends, 60 to 70 μ in length; ascospores in two groups of four spores each, two-celled, slender, with pointed ends, slightly ridged at transverse septum; 50 × 4.5 μ; ascospores liberated by dissolution of the ascus wall and held together somewhat in groups of 4 by motionless flagellae; flagellae 50 to 100 μ in length; arthrospores of non-gemetic origin, spherical to ampulliform, 25 μ in diameter. Two other cell forms also found: (1) much elongated filamentous cells; (2) elliptical or ovoid cells, gametic in function, new cells formed in bipolar direction by apical budding and also

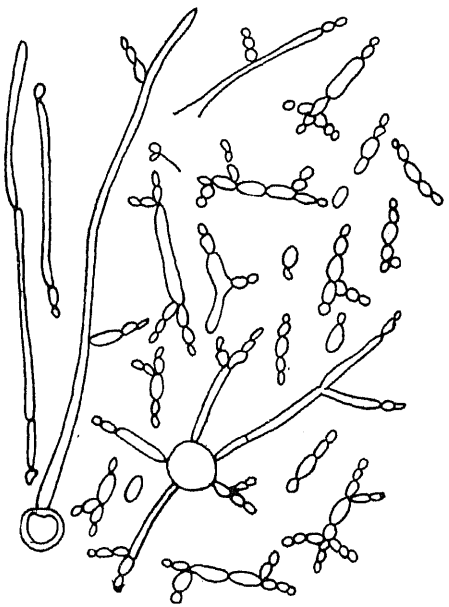


Fig. 138–A. — *Nematospora Lycopersici.* Showing the Shape of the Cells and the Formation of Daughter Cells (after Schneider).

[1] Schneider, A. A parasitic saccharomycete of the tomato. Phytopathology 6 (1916) 395–399. Further note on a parasitic saccharomycete of the tomato. Phytopathology, 7 (1917) 52–53.

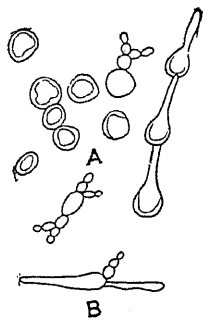


Fig. 138–B. — Arthrospore Formation in *Nematospora Lycopersici.*

A, An Ascospore Beginning a New Vegetative Cycle; *B*, (after Schneider).

by apico-lateral budding at cell unions. The elliptical and ovoid cells alone are gametic in function." Apparently this yeast is a parasite on the ripe fruit of *Lycopersicum esculentum.*

Genus XV. Coccidiascus. Chatton

Budding cells, ascs seem to be derived from an isogamic copulation with eight ascospores.

COCCIDIASCUS LEGERI. Chatton [1]

This yeast was observed in a Muscide *Drosophila funebris.* The yeast infects the cells of the middle intestine and lives in the vacuoles of the cells in which it multiplies by ordinary budding and also by ascs. Budding constitutes the intracellular multiplication while ascs are the external agents of propagation. The parasite possesses the form of a yeast and never produces a mycelium. Formation of ascs seems to be preceded by an isogamic copulation. The ascs have the shape of bananas. They contain 8 ascospores, the special shape of which makes this yeast resemble *Monospora cuspidata* and *Nematospora coryli.*

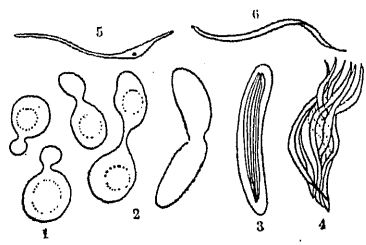


Fig. 138–C. — *Coccidiascus Legeri.*

1, Vegetative cells. 2, Copulation. 3, Ascs. 4, Ascospores Detached from Asc. 5–6, Ascospores (after Chatton).

[1] Chatton. Coccidiascus Legeri. nov. gen. n. sp. Levure ascospore parasite de cellule intestinale de Drosophila funebris. Comp. Rend. Soc. Biol. 75, 1913.

CHAPTER XI

THE NON-SACCHAROMYCETES OR DOUBTFUL YEASTS

THIS is a provisional group in which one may place all yeasts which do not form ascospores and whose places in classification are uncertain.

Genus Torula. Turpin

Under the name Torula,[1] Hansen has grouped a large number of asporogenic species often capable of fermenting sugars, among which are some which do not form a scum and others which form a ring around the culture flask or scum without the interposition of air, analogous to those which are produced by the Saccharomycetes of the third group. The greater number of the *Torulae* possess regular spherical cells and contain a globule of fat which gives them a special characteristic. They may, then, be regarded as asporogenic *Torulaspora*. But some are oval or elongated and offer the appearance of ordinary yeasts. A certain number of them form a red, black or brown pigment. The formation of these pigments seems to depend, at times, on the presence of silver in the medium. Light sometimes represses the production of pigments. The investigations of Chapman seem to indicate that the coloring matter in red torula is due to some other substance along with carrotene. The *Torula* are very widespread in nature. They are encountered in the soil, breweries, insects, milk, in secretions of trees, etc.

A. TORULA FROM BREWERIES AND OTHER SOURCES

HANSEN'S TORULA

Hansen[2] has described a number of *Torula* which we shall now consider.

Torula No. 1. It appears in beer wort as isolated cells or as colonies formed by a small number of cells. The cells are 1.5 to 4.5 μ in diameter and often have a large vacuole with a large refractive body. This yeast forms a small amount of alcohol in beer wort and does not secrete invertase.

[1] This name leads to confusion for it is also applied to the Mucedinaceae, very different from the yeasts (genus Torula of Persoon); in spite of this the name is commonly used.

[2] Hansen, E. C. Sur les Torula de M. Pasteur. Comp. Rend. des trav. du lab. de Carlsberg, 2, 1888.

Torula No. 2. This yeast differs from the preceding by its larger cells, from 3 to 8 μ in diameter, and granular protoplasm.



Fig. 139. — *Torula* 6 of Hansen. Growth of Cells in the Sediment in Beer Wort after One Day at 25° C. (after Hansen).

Torula No. 3. Morphologically similar to the preceding yeast, this yeast produces 7 to 8 per cent of alcohol by volume in beer wort. It forms a foam and sets free much carbon dioxide. No invertase is produced.

Torula No. 4. It possesses cells from 2 to 6 μ in diameter. It inverts saccharose and gives a little more than 1 per cent of alcohol by volume in beer wort with the formation of much foam. It does not ferment maltose.

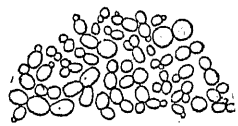


Fig. 140. — *Torula* 7 of Hansen. Growth of Cells in Sediment in Beer Wort after One Day at 25° C. (after Hansen).

Torula No. 5. This yeast resembles *Torula No.* 1 in the shape and dimensions of its cells. It forms a homogeneous dull gray scum on beer wort at the temperature of the laboratory and in other liquids containing 10 per cent of alcohol. In solutions of saccharose, it forms a thin scum. It inverts saccharose but produces only traces of alcohol in sugar solutions.

Torula No. 6. It possesses spherical or oval cells. The limits of temperature for budding are 4–6° C. and 36–37° C. (Fig. 139.) This species causes an apparent fermentation in beer wort and yields 1.3 per cent of alcohol. It produces no fermentation in maltose solutions. It inverts saccharose and forms 5.1 to 6.2 per cent of alcohol by volume in yeast water with added sugars at 25° C. after 15 days; after two months, seven per cent of alcohol is formed. In solutions of dextrose, it produces 6.6 to 8.5 per cent of alcohol by volume.

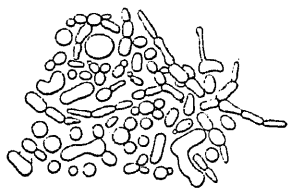


Fig. 141. — *Torula* 7 of Hansen on Scum at the End of 10 Months in Wort (after Hansen).

Torula No. 7. Found in the soil under vines, this species is made up of ordinary oval cells larger than those in the preceding yeast. (Fig. 140.) The oval cells are irregularly formed. (Fig. 141.) The temperature limits for budding are 0.5 and 38–39° C. This species produces 1 per cent of alcohol by volume in beer wort. It does not ferment maltose or invert saccharose. In yeast water with dextrose added (10 or 15 per cent) it yields at the end of 15 days, 4.5 per cent of alcohol by volume. After 28 days, it produces 4.8 to 5.3 per cent of alcohol. Hansen thinks that this species like the preceding participates in the fermentation of wine and cider.

WILL'S TORULA

Will[1] has isolated from the cooling apparatus and from the air in breweries, etc., seventeen forms of *Torula* as follows:

Will's Torula No. 1. This species produces a thin, white, dull scum and a very evident ring. It forms a very evident deposit at the bottom of the culture flask. The scum and ring appear at the end of the third day. The thermal death point of this yeast in beer wort and water is 65° C. The cells are very small, circular in shape like the typical *Torula*. A few elongated and sausage-shaped cells may also be found.

Will's Torula No. 2. The scum and ring look like those in Will's *Torula No.* 1. The cells are oval and sometimes very elongated in the form of a sausage. This species perishes at 60° C. in must and water.

Will's Torula Nos. 3 and 4. These species form a very thick folded scum, of a whitish yellow color, and also a well-developed ring. At the bottom of the culture flask there is an abundant sediment. The scum develops rapidly and covers the surface of the culture three days after inoculation. The cells are shaped like the typical *Torula*. These two species are almost identical. They are distinguishable only by the degree of changes in culture media. Both are killed at 60° C. in must and water.

Will's Torula No. 5. This species is most often round or oval. It yields a very thin scum of small islands and a slight ring. The sediment is mucous and well developed. Under certain conditions this yeast gives on beer wort a flowing appearance. The cells are killed at 65° C. in must and water.

Will's Torula No. 7. The cells are spherical with a spongy interior and a very distended membrane. An abundant deposit is formed at the bottom of the culture flask and on the surface a very mucous scum. In cultures which are a little old, it forms a thick mucous sediment filling the entire volume of wort which is changed into a compact mass. The thermal death point is around 60° C. in wort and water.

Will's Torula No. 8. The cells are large, oval and often elongated. A well-developed ring is produced, a feeble scum and a mucous irregularly developed sediment at the bottom of the culture flask. The thermal death point in beer wort and water is 60° C.

Will's Torula No. 9. The cells are very small and oval with some elongated to the shape of a sausage. A feeble ring is formed,

[1] Will, H. Beiträge zur Kenntniss der Sprosspilze ohne Sporenbildung. Zeit. f. d. Ges. Brauw. 26, 1903; 19, 1907; Cent. Bakt. 17, 1907; 21, 1908.

a well-developed scum, forming small islands, and an abundant deposit. The thermal death point is 65° C. in beer wort and water.

Will's Torula No. 10. The cells of this species are similar to those of No. 9. A powerful ring is formed, a well-developed scum and a rich sediment. The thermal death point is about 60° C.

Will's Torula No. 11. The cells are small, spherical and oval. A ring is produced with a folded scum of a white color. At the end of 3 days, the surface of the culture fluid is covered with this scum and at the bottom of the flask there is an abundant sediment. This yeast succumbs at 65° C. in must and water.

Will's Torula No. 12. This yeast is composed of elongated, or fusiform cells closely resembling those of *Mycoderma.* There are also present a few rounded and oval cells. A strong scum much folded of a light yellow or brown color is produced. It may become a pale red after three months. This scum resembles that of *Mycoderma* very much. It appears soon as little floating islands which look like fat globules. The thermal death point in beer wort and in water is 60° C.

Will's Torula No. 15. The cells have different shapes, most often elongated, oval or shaped like a sausage. The scum is well developed, very much folded and resembles that of *Mycoderma.* It has a white, slightly yellow, color. It begins to appear at the end of three days. The cells succumb at 65° C. in must and in water.

Will's Torula No. 16. It is made up of spherical or oval cells mixed with long cells. The scum is well developed, compact and of a yellowish white color. A mediocre amount of sediment is formed. The scum appears on the third day. The thermal death point in must and water is 60° C.

Will's Torula No. 17. The cells are small, quite round and possess the typical *Torula* shape. The scum is moderately developed, compact and white. The thermal death point in must and water is 60° C.

The greater number of these *Torula* are not distinct species. More often they are of simple varieties or different races. All grow at low temperatures on beer wort. The minimum temperature for budding is 5–6° C. The optimum temperature is 30–35° C. Almost all of them impart to beer wort an agreeable fruity odor. Species Nos. 7 and 8, however, produce a disagreeable odor.

Will [1] has recently described a number of other species found in and about breweries. They have been characterized as follows:

[1] Will, H. Beiträge zur Kenntniss der Sprosspilze ohne Sporenbildung welche in Brauereibetrieben und in deren Umgebung vorkommen. Cent. Bakt. Abt. 2, 46, 1916.

Torula Nos. 3 and 4. Cells spherical (3–4 μ). The giant colonies are thick with a flat edge which is sinuous. It ferments dextrose, levulose, galactose and raffinose. A scum is formed on liquid media, dry and white at first, later changing to a brownish yellow. The temperature limits for growth on wort are 0.5 and 33° C. It was found in the air about breweries.

Torula No. 11. The cells are spherical or ellipsoidal (3–4 μ). A scum is formed in liquid media which is dry and folded. It is of a gray color. The giant colony exists in the form of a flat layer with a united edge. It ferments dextrose, levulose, galactose and saccharose. The temperature limits for growth on beer wort are 0.5 and 33° C. It was isolated from wort.

Torula No. 17. The cells are spherical and sometimes ellipsoidal. (About 3 μ in diameter.) A thin dry scum is formed in liquid media of a flat white or yellow color; it is slightly folded and grips the sides of the culture flask. The giant colonies are round. It ferments dextrose, levulose, galactose, saccharose, lactose and raffinose. It was isolated from brewing water.

Torula No. 6. The cells are spherical, sometimes ellipsoidal. They develop in liquid media, especially in the form of a sediment. A very thin scum appears quickly. The giant colonies are like those of *Torula No.* 2. It ferments dextrose, levulose, galactose, saccharose and maltose. The temperature limits for growth on beer wort are 0.5 and 30° C.

Torula No. 5. The cells are spherical, ellipsoidal or elongated. A scum and ring are formed on liquid media. The scum is colorless, moist and thin. Dextrose, levulose, saccharose, galactose, maltose and raffinose are fermented. The temperature limits for growth are 0.5 and 35° C. It was isolated from wort.

Torula No. 7. The cells are spherical and at the beginning develop at the bottom of the liquid. A ring and a scum are finally formed. The ring is solidly adherent and of a gelatinous consistency. The scum is well developed, mucous and, at first, uncolored. The giant colony is a reddish brown at first, finally a brown. It ferments dextrose, levulose, galactose, saccharose, maltose, lactose, raffinose and arabinose. The temperature limits are 2° C. and 30° C. It was isolated from the air.

Torula No. 8. The cells are spherical or ellipsoidal, slightly pointed. They become elongated at low temperatures and may reach 5 μ. In liquid cultures there is feeble development as a sediment but a scum and a mucilaginous ring are formed. The giant colonies are a deep brown. It ferments dextrose, levulose, galactose, maltose, lactose, raffinose and arabinose. The temperature limits are 0.5 and 35° C.

Torula No. 9. The cells are ellipsoidal, sometimes in the shape of lemons. There is a ring formed but no scum. The giant colonies are flat and transparent. It ferments dextrose, levulose, galactose, maltose, lactose, raffinose and arabinose. The temperature limits are 0.5 and 40°. It was isolated from brewing water.

Torula No. 1. The cells are spherical or ellipsoidal. On liquids the development is rapid with a superficial vegetation, at first in the form of a ring, and later in a flat and white scum. It ferments dextrose, levulose, saccharose, maltose, lactose, raffinose and arabinose. The temperature limits are 2° and 40° C. It was isolated from brewing water.

Torula No. 2. The shape and size of the cells of this yeast are variable, in general, spherical or ellipsoidal. There are many giant cells in the cultures. On liquid media it develops like the former yeast. The giant colonies have a sort of crater in their centers and a thin marginal part. It ferments dextrose, levulose, galactose, saccharose, maltose, raffinose and arabinose. The temperature limits are 0.5 and 39° C.

Torula No. 10. The cells are ellipsoidal or elongated. On liquid media, the development is slow in the beginning with a sedimental growth but finally a white or rose scum is formed. Gelatin is rapidly liquefied. The yeast ferments dextrose, levulose, galactose, saccharose, maltose, lactose and arabinose. The temperature limits are 2° C. and 35° C.

Torula No. 15. The cells are spherical or ellipsoidal. It develops slowly on liquid media with a superficial vegetation. The scum is dull white in the beginning, then yellow. It ferments dextrose, levulose, galactose, saccharose, maltose, lactose, raffinose and arabinose. The temperature limits are 0.5 and 35° C.

Torula No. 16. The cells are spherical and sometimes ellipsoidal. They grow rapidly on liquid media. A yellowish brown scum is formed. It ferments dextrose, levulose, galactose, saccharose, maltose, lactose, raffinose and arabinose. The temperature limits are 0.5° C. and 30° C.

Torula No. 12. The cells have a very variable appearance. They are either round or ellipsoidal and small. It develops on liquid media, almost always in the form of a scum, at first white, later becoming yellow. The giant colonies are reddish in their centers and white around the periphery. It ferments dextrose and levulose actively and maltose, galactose and saccharose feebly. The temperature limits are 2° and 35° C.

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TORULA OF LINDNER AND MEISSNER

Lindner [1] has described two *Torula* in detail from the collection at Berlin. They are *Torula* Nos. 63 and 64. Their cells often reach the size of beer yeast and present a very granular protoplasm. The first of these two species forms a cartilaginous scum on beer wort, difficult to crush under the cover slip with a moist and transparent appearance. On wort gelatin in streaks, it produces a mucous deposit, sometimes cartilaginous. Under the same conditions, the second variety produces a mucous sediment. The cells of both species rarely contain fat globules and possess a thick membrane, the exterior membrane of which shows a tendency to detach itself. Eleven forms of Torula have been isolated by Meissner.[2] They cause a defect in wine in which it becomes thick and greasy.

TORULA NOVAE CARLSBERGIAE. Grönlund [3]

This species has cells of various shapes and gives a disagreeable taste to beer wort. According to Schjerning, it inverts saccharose and sets up an alcoholic fermentation in solutions of saccharose, dextrose and maltose. In beer wort, it is able to reproduce about 4.7 per cent of alcohol by volume.

TORULA BRETTANOMYCES. Clausen

Under this name, Clausen [4] described a special group of *Torula* which produced a secondary fermentation in English beers. It differs from other *Torula* in that, if a preliminary fermentation is carried on by *Saccharomyces*, it will multiply and ferment the remaining sugar. It forms acids which combine with the alcohols to form aromatic substances giving the beer a special flavor and aroma. Schiönning [5] has studied this group. The *Torula* which make up this group ferment maltose actively. In beer wort with about 10 per cent saccharose, they form about 10 per cent of alcohol by volume but the fer-

[1] Lindner, P. See reference for Willia belgica.

[2] Meissner, R. Studien über das Zähnwerden von Most u. Wein. Landw. Jahrb. 27, 1898.

[3] Grönlund, Ch. En ny Torula-Art og to nye Saccharomyces Arter. Vidensk. medd. fra den Natur. Foren. Copenhagen, 1892.

[4] Clausen, H. Occurrence of Brettanomyces in American Lagerbeer. Amer. Brewing Rev. 19, 1905.

[5] Schiönning, H. On Torula in English Beer Manufacture. Comp. Rend. trav. lab. de Carlsberg, 7, 1908.

mentation is accomplished slowly and only ends after eight months at 20° C. They ferment sucrose without giving products which reduce Fehling's solution, although an inversion of the saccharose is brought about. According to Schiönning, these types may be distinguished in these ways. *Torula A* may be distinguished from *Torula B* by its action towards sugars and also by the temperature limits for budding.

Torula A. The cells are ellipsoidal while some are sausage shaped or in the form of a mycelium. Others present odd, irregular shapes. The size of the cells is variable. Giant cells are found with a very refractive protoplasm, showing vacuoles scarcely visible in which a mobile granule is often found. The temperature limits for budding are 5–7° C. and 40–40.5° C. The races of type A produce, in old liquid cultures, a loose scum in which the cells are filamentous, resembling a mycelium. It ferments dextrose, levulose, saccharose and maltose (saccharose more actively and maltose less actively than type B). It has no action on lactose and dextrine.

Torula B. The cells resemble somewhat those of *Torula A.* They are often sausage shaped. Long mycelial structures are found among them. The temperature limits for budding are 3–4° C. and 39–39.5° C. In old liquid cultures, the races of type B form a scum identical with that of *Torula A.* They ferment saccharose, maltose, dextrose, levulose and lactose but do not act on dextrose.

BEIJERINCK'S TORULA

Beijerinck [1] has isolated three yeasts which do not sporulate and which ought to be classed among the *Torula.* They are:

Saccharomyces fragrans, Beijerinck. According to Beijerinck, this yeast is a contamination in compressed yeast. It has been insufficiently described.

Saccharomyces muciparus, Beijerinck. Related to the preceding species, this is also incompletely known. It is characterized by a very evident polymorphism. In old cultures, it presents filamentous and yeast forms.

Saccharomyces curvatus, Beijerinck. This yeast was isolated by Beijerinck from an impure culture of *S. muciparus.* It seems to correspond to the cheesy yeast described by Pasteur. The shape is much like that of *Saccharomyces Pastorianus.* It is curved, however, whence the name *S. curvatus.* This yeast has the characteristic, as Pasteur found, of not being broken up in water. It settles to the bottom of the culture flask as a curd leaving the supernatant liquid

[1] Beijerinck, M. W. Die Erscheinung des Flokenbildung oder Agglutination bei Alkoholhefen. Cent. Bakt. 20, 1908.

almost clear. According to Beijerinck, this yeast seems to possess the ability of agglutinating itself.

TORULA COLLICULOSA. Hartmann

This species was isolated from a sample of yeasts secured from Java by Hartmann.[1] It was associated with *Mucor amylomyces*. It presents the appearance of ordinary *Torula*. Its cells are spherical and contain a fat globule. Its ordinary dimensions are 3.5 μ but they may vary between 1.7 and 9.7 μ. Budding is accomplished on all sides of the cell. Frequently two or three buds appear simultaneously. The cells are often united in chains of two or three, the mother cell being much larger than the daughter cell. The optimum temperature for budding on wort agar is situated between 25° C. and 30° C. Budding stops at 7° C. and 45° C.

The giant colonies obtained on beer wort agar have a characteristic aspect. They form a prominent wart. On beer wort gelatin, the colonies develop well and liquefy the gelatin after eight weeks. Cultivated in beer wort at 25° to 30° C., *Torula colliculosa* produce, at the end of 24 hours, a powdery white sediment. After 40 hours the liquid clarifies itself. Depending on their age, the cells act differently on maltose. The oldest cells are able to ferment this sugar while the young ones have no action. This species ferments dextrose, raffinose and saccharose.

SACCHAROMYCES SPEC. Saito

Isolated from "koji" by Saito,[2] this yeast forms on decoction of "koji" or on beer wort globular or oval cells, without color, about 4 to 7 μ in diameter. The cells are usually isolated and show one or many vacuoles and a finely granular protoplasm. In old cultures they often take the form of a sausage. On gelatin plates, the colonies first appear as little round points, later changing to a white mass in the shape of a dome with an uneven surface. On the surface, they appear as numerous spots made up of elliptical cells. This species seems to be related to *Saccharomyces awamori*. It ferments dextrose, levulose, d-galactose, saccharose, maltose, melibiose, raffinose and α-methylglucoside. It has no action on inuline or lactose. In wort after 20 days, it produces 5.99 per cent of alcohol by volume.

[1] Hartmann, M. Eine rassespaltige Torula-Art welche nur zeitweise Maltos zu vergären vermag. Wochenschr. f. Brau. 20, 1903.

[2] Saito, K. Notes on Formosan fermentation organisms. The Botanical Magazine, 15, Nos. 21 and 52, 1902.

SACCHAROMYCES AWAMORI. Inui

This species was found by Inui [1] who isolated it from foamy wine of the Japanese called awamori. It produces on gelatin an irregular colony. It is able to stand three hours' exposure at 50° C. It develops in liquids containing 8 per cent of alcohol but growth is stopped by 20 per cent. In beer wort, it endures 6 per cent of alcohol.

DE KRUYFF'S TORULA

De Kruyff [2] isolated, from the soil and living and dead leaves in Java along with *Zyg. javanicus*, seven species of yeasts under the name of *Saccharomyces javanicus* 1–7. These are ellipsoidal or round yeasts all of which, with the exception of one, ferment saccharose, dextrose and maltose. As no sporulation has been described for them they may be regarded as *Torula*.

TORULA OF PEARCE AND BARKER

Pearce and Barker [3] have isolated a series of Torula from cider in England.

Yeast C. This is a yeast with small, spherical or oval cells ($4.5 \times 3.7\,\mu$). The maximum temperature for budding is 37–37.5° C. On gelatin plates, it forms damp, transparent, round colonies. On streaks, it gives a moist vegetation with indented rugose edges.

Yeast D. The cells of this yeast are generally oval ($4.5 \times 3.7\,\mu$), sometimes elongated and shaped like a sausage. The maximum temperature for budding is 32° C. The cultures on gelatin plates are round and dry with a tendency to flatten onto the gelatin. The edges are irregular and festooned. On streaks the growth becomes slightly fringed at the edges. This species forms a scum on all sugar media but does not cause a fermentation.

Yeast E. The cells are oval shaped ($11.9–6.8 \times 3.4\,\mu$). The maximum temperature for budding is situated near 38° C. This species forms a well-developed scum on solutions of maltose. It does not produce fermentation.

[1] Inui, T. Untersuchungen über die niederen Organismen welche sich bei der Zubereitung des alkoholischen Getränks Awamori beteiligen. Jour. of the College of Sciences. Univ. Tokyo. 15, 1901.

[2] De Kruyff, E. Untersuchungen über auf Java einheimische Hefenarten. Cent. Bakt. 21, 1908.

[3] Pearce, B., and Barker, P. The yeast flora of bottled ciders. The Journal of Agricultural Science, 3, 1908.

Yeast J. The cells of this yeast are oval and spherical (8.5–6.8 × 3.4 μ). The maximum temperature for budding is situated between 30 and 32° C. Colonies on wort gelatin in plates are round, moist and partly transparent. On streaks, the growth is flat and spreading. This species ferments dextrose, levulose and saccharose.

Yeast L. This species has small cells shaped like a sausage (12–6.8 × 2.7 μ). The maximum temperature for budding is about 38° C. Colonies on wort gelatin in plates are spherical with thin edges. On streak cultures vegetation is compact. This species ferments dextrose and maltose.

Yeast M. The cells are oval (8–6.8 × 5 μ). Sometimes they are sausage shaped. The maximum temperature for budding is situated between 35 and 28° C. On wort gelatin in plates, the colonies are dry, spherical and with a solid appearance. Gelatin is liquefied after a certain time. This species ferments dextrose, levulose, maltose and saccharose.

SACCHAROMYCES BRASSICAE I. Wehmer

Isolated by Wehmer,[1] from fermentation of sourkraut, this species presents spherical or elongated cells closely resembling those of *S. cerevisiae* but smaller (4–6 × 5 μ). The development on agar and gelatin with a decoction of kraut, in stab and streak, gives firm, grayish white, slightly raised colonies. The sediment in a fermenting liquid forms a grayish white mass from which escape bubbles of gas. This yeast produces an active fermentation in decoctions of kraut, especially that to which dextrose is added. It ferments beer wort.

SACCHAROMYCES BRASSICAE II. Wehmer

This yeast was isolated by Wehmer from the same source as the preceding one. The cells are almost spherical and do not exceed 3.6 to 4.8 μ in diameter; often they are much smaller. They possess very refractive granules in their vacuoles. This species has the same appearance on gelatin. In a decoction of kraut in dextrose solutions and in beer wort, it produces a fermentation.

SACCHAROMYCES BRASSICAE III. Wehmer

Isolated also from sourkraut, this species possesses ellipsoidal cells, slightly elongated and quite small (7 × 4 μ in diameter). The appearance of the cultures on gelatin is quite different from that of the two preceding yeasts. This yeast produces an alcoholic fermentation.

[1] Wehmer, C. Untersuchungen über Sauerkrautgärung. Cent. Bakt. 2, 1905.

TORULA HOLMII. (Holm) Jörgensen [1]

Syn.: TORULA A. Holm

This yeast was isolated by Holm in Jörgensen's laboratory. The growth in the sediment of young cultures contains small, oval cells. Along with these are found large cells, oval or round. The length of the cells varies from 3.5 to 5.5 μ and their width from 1.4 to 2.1 μ. In must this species produces a feeble fermentation after which the alcohol content may reach 0.32 per cent by volume. It inverts saccharose and raffinose and ferments the invert sugar. However, it has no action on maltose, dextrose and dextrine. At the end of three to five days at 25° C., it forms a scum on beer wort, the cells of which are round or oval. In yeast water to which dextrose has been added the cells of the scum look like those of *S. Pastorianus*. They may also be irregular. The surface colonies on gelatin with 10 per cent wort are round, white, shiny, slightly bulged and with entire edge.

TORULA THERMANTITONUM. Johnson

Discovered on the leaves of the Eucalyptus and studied by Johnson [2] and Hare, this species possesses cells of small size which are egg shaped. It is of special interest on account of an abnormal resistance to high temperatures. The temperature limits for budding are situated in the vicinity of 10° C. and 84° C. The optimum is between 40 and 44° C. According to Lindner, it is a brewery yeast which induces a bottom fermentation and a good clarification. It ferments dextrose, levulose, saccharose, maltose, dextrine, d-mannose, d-galactose, raffinose, α-methylglucosides, xylose and inuline but has no action on lactose and melibiose.

TORULA FROM VACCINE PULP. Lesieur and Mangini

Lesieur and Mangini have found the presence of yeasts in samples of vaccine pulp. These yeasts belong to the genera *Mycoderma* and *Torula*.

Torula I. The cells are round, more often spherical. On wort at 25° C., the yeast causes a cloudiness after 40 hours, and very slight scum formation. After five days, there is visible scum and sedimental growth at the bottom of the container. The yeast grows a little

[1] Jörgensen, A. Die Mikroorganismen der Gärungsindustrie, Berlin, 5th Edition, Paul Parey, 1909.

[2] Johnson, G. Saccharomyces Journal of the Institute of Brewing, 11, 1905.

at 14° C. The optimum temperature for growth seems to be situated between 25° C. and 37° C. At 37° C. the yeast vegetates but stops at 40° C. This yeast ferments levulose. The inoculation of a guinea pig gave negative results.

Torula II. The cells are variable in shape, either spherical or oval (5–5.5 μ in diameter). On Raulin's gelatin, the cells are round and there are rudiments of a mycelium. On liquid media, there is a sediment and after 10 to 12 days a slight ring appears. Growth begins at 14° C.; the optimum temperature is between 19 and 20° C.

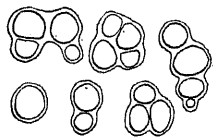


Fig. 141–A. — *Torula* III from Vaccine (after Mangini).

Torula III. The cells are ordinarily spherical, rarely oval (4–6.5 μ). On potato, the cells are united into groups of 2, 3, or 4 individuals, rarely more. There is a gelatinous substance resembling a zoögloea. On liquid media an abundant sediment and a white scum are formed after 11 days. This yeast develops at 14° C. The maximum temperature for budding seems to be situated between 25° and 37° C. The species is able to grow at 45° C. There is no fermentation and it is non-pathogenic.

Olsen-Sopp have isolated from Taette along with *Saccharomyces taette major* and *minor* some *Torula* which form small round or oval colonies which are distinguishable from each other by their action toward sugars.

TORULA OF ROSE

Rose [1] has isolated two types of *Torula* from the mucous secretions of oaks. Both have round cells (5 μ in diameter) and act as bottom yeasts. One, *Torula A*, ferments dextrose, trehalose, raffinose inuline, α-and β-glucosides and possibly melibiose. The other, *Torula B*, acts like the *Torula A* and differs only by the fact that it does not act on inuline and melibiose.

WEHMER'S TORULA

Wehmer [2] has isolated from pickle brine a small round *Torula* which vegetates in nutrient solutions containing 10 to 15 per cent of salt. In solutions of 21 per cent of salt it remains capable of developing for months. It seems to originate from sea water or the herring.

[1] Rose, L. Beitrage zur Kenntniss der Organismen in Eichenschleimfluss. Inaugural dissertation at the University of Berlin. June 1910.

[2] Wehmer, C. Zur Bakter. und Chemie der Heringslake. Cent. Bakt. 3, 1897.

TORULA SP. Saito [1]

This yeast possesses spherical cells with resistant walls with a number of fat globules in the protoplasm. The cells form a mucilaginous sediment. On a fat substrate they become oval or elongated. The giant colonies give a mucilaginous stratification of a yellow color. In wort or decoction of "koji," this yeast causes a feeble escape of gas; at first, there is a cloudiness produced, later, the formation of a united scum. This species is closely related to the preceding one and possibly identical.

HÖYE'S TORULA

Höye [2] found many species of *Torula* in solutions to which salt had been added. These will be described.

Salt Yeast B, Höye. This is a yeast with round or oval cells, the shape varying somewhat with the concentration of salt. When 15 per cent of salt is added, the cells become more oval than with lesser amounts. With 20 per cent of salt the cells become more pointed, resembling the tubercles of beets. The membranes of the cells often have short points to the number of two or three on each cell. Frequently the cells are joined together by means of these points. This yeast does not grow in potato wort.

Salt Yeast, Höye. In fish bouillon with 15 per cent of salt added, this species possesses cells of various shapes. The structure is often moniliform. In 25 per cent of salt, the cells are oval and located in chains. In 35 per cent of salt, they become round. This yeast does not develop in potato wort. Schultz has mentioned yeasts of the ellipsoideus type from brines used to preserve legumes.

Recently, Coupin has found in sea water a *Torula* which does not grow in media unless salt is added to it. Kita has found two species of *Torula* which develop in beer wort with 20 per cent of added salt. They grow easily in solutions with maltose but not in those which contain dextrose.

[1] Saito, K. Mikrobiol. Stud. über die Soya-Bereitung. Cent. Bakt. 17, 1906.

[2] Höye, K. Recherches sur la moisissure de Bacalao et quelques autres Mikroorganismes halophiles. Bergens. Museum Aarbog. No. 12. 1906.

B. TORULA FROM MILK

TORULA KEPHIR. Heinze and Cohn [1]

Syn.: SACCHAROMYCES KEPHIR. Beijerinck

Found by Beijerinck [2] in képhir, this yeast possesses cells of variable shape, generally elongated, developing on nutrient gelatin with slightly winding colonies and not liquefying the gelatin. According to Beijerinck it secretes lactase. Schurmans-Stekhoven, however, have not been able to confirm this fact. It inverts saccharose but has no action on maltose.

SACCHAROMYCES TYROCOLA. Beijerinck

This species was isolated by Beijerinck [3] from Eidam cheese. It is a yeast with small cells, round, giving snow-white colonies on gelatin. As with the preceding species, it inverts lactose and saccharose but does not act on maltose.

FREUDENREICH'S KEPHIR YEAST

This species was found by Freudenreich [4] in various samples of kephir. It gives a vigorous growth and feeble fermentation in beer wort but does not seem to cause any fermentation in milk. The growth consists of oval cells (3.5 μ in length and 2–3 μ in width). It does not form a scum. The optimum temperature for budding is 22° C. According to Freudenreich, this species is able by a symbiosis with *Dispora causica* and *Streptococcus* α and β to bring about a fermentation in milk. It does not act on lactose when it is isolated by itself.

DUCLAUX'S TORULA [5]

This yeast was isolated from milk in which it produced an active fermentation. It is a small, ovoid yeast. Packets which may reach large size are formed by budding. Inoculated into gelatin, it gives little growth and this is almost entirely on the surface. In the same

[1] Heinze, B., and Cohn, E. Ueber Milchzuker vergärende Sprosspilze. Zeit. Hygiene. 46, 1908.

[2] Beijerinck, M. W. Sur le Kephir. Arch. neerlandaises des Sc. exactes et nat., 23, 1889.

[3] Beijerinck, M. W. Die lactase ein neues Enzyme. Cent. Bakt. 6, 1889.

[4] Freudenreich, E. Bakterien Untersuchungen über den Kefir. Cent. Bakt. 3, 1897.

[5] DuClaux, E. Fermentation alc. du sucre de lait. Ann. Past. Inst. 1, 1887.

medium with 1 per cent of glycerol added, it produces a button-shaped growth and a little development along the line of inoculation. It is separated from the yeast of Adametz, which will be mentioned later, by the fact that its branching structure is less developed. In beer wort, similar development is secured. The cells elongate and become cylinders (7 μ long). The yeast grows easily in milk and neutral serum and develops especially in liquids exposed to the air and with difficulty in the bottom of flasks. The aerobic life does not hinder its enzyme activity. This species secretes neither rennin nor casease but does produce lactase. The optimum temperature for fermentation is between 25 and 30° C. The thermal death point is 50° C. for cells in the dry state and 60° C. for moist cells. No coagulation is secured in milk. An alcoholic drink slightly acid and with an agreeable taste is formed from milk. It ferments easily lactose, dextrose, d-galactose, saccharose and more difficultly maltose.

TORULA LACTIS. (Adametz) Heinze and Cohn

This species was isolated from a spontaneous fermentation in milk by Adametz.[1] It has oval or elliptical cells (7–10 $\mu \times 5$), sometimes spherical (3–4 μ), larger than those of the preceding species. The buds are usually formed simultaneously at both of the opposite ends of the ellipse. On the plaster block, this yeast forms no ascospores but the cells continue to bud and elongate. On peptone gelatin it does not do well and gives almost entirely a superficial growth. The colonies are round with slightly branching edges and of a brown color. On peptone gelatin with one per cent of glycerol added, in stab cultures, this yeast forms, like the preceding, a superficial button along the inoculation growth from which, in fifteen days, fine branches extend. On beer wort gelatin, an abundant surface growth is secured in which the center is slightly raised. In sterile milk fermentation phenomena are presented in 24 hours at 40° C., in 48 hours at 38° C., and in 4 days at 25° C. No rennin or casease is formed. In beer wort it grows as a sediment at the bottom of the flask and induces a manifest fermentation. It starts a faint fermentation in milk after 24 hours a little less vigorous than that caused by the *Torula* of Duclaux. It ferments maltose with difficulty but ferments easily lactose, d-galactose, dextrose and saccharose (as quickly lactose as saccharose). The thermal death point of dry cells is between 50 and 60° C. and of moist cells 56° C.

[1] Adametz, L. Saccharomyces lactis, eine neue Milchzucher vergärende Hefenart. Cent. Bakt. 5, 1889.

KAYSER'S YEAST

Secured from milk at a farm in Brie, Kayser [1] found that this yeast possessed cells from 6 to 8 μ long and 3 to 5 μ wide. It forms neither casease nor rennin. Like the two preceding species, it ferments saccharose and lactose (the lactose as easily as the saccharose), d-galactose, and dextrose but acts with difficulty on maltose. The thermal death point is 55° C. for moist cells and 90–100° C. for dry cells. On gelatin, it looks like the preceding yeast and is distinguished only by the fact that the fibrous appearance is less pronounced.

TORULA COMMUNIS. Browne

Browne [2] has found a *Torula* the most abundant organism in raw sugar from Cuba. A similar organism was also found in raw sugar and soft refined sugars from the British West Indies. Owen [3] has also mentioned a similar organism. The colonies on raw sugar agar,

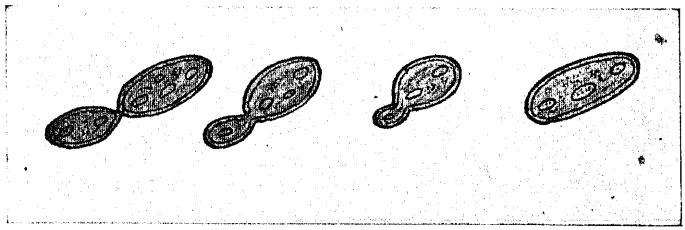


Fig. 141–B. — Magnified Cells of *Torula communis* (after Browne).

according to Browne, appear first as small white cysts which are pointed under the microscope. These cysts increase in size to a diameter of 0.2–0.5 mm. until they reach the surface of the agar after which they spread out in all directions. The colony gradually assumes a circular shape from 3–10 mm. in diameter and is grayish white in color. Old colonies are brownish in color. Under high power of the microscope, no mycelium is seen. The cells are separate and look like yeasts. *Torula communis* grows readily in all concentrations of sugar solutions. A granular deposit is formed and, after a time, a thin marginal scum. There seems to be slight evolution of gas. No froth or foam is formed as is often present with strongly fermenting organisms. The action on raw sugar seems to be a destruction of the

[1] Kayser, E. Contribution physiologique des levures alcooliques de la lactose. Ann. Past. Inst. 5, 1891.

[2] Browne, C. A. The Deterioration of Raw Cane Sugar. A Problem in Food Conservation. J. Ind. Eng. Chem. 10 (1918), 178–190.

[3] Owen. Louisiana Planter, 56, 173.

invert sugar, fructose being the constituent most strongly attacked. Sucrose is not inverted. Data are produced by the author to show that this organism is the strongest fermenter between the ninth and fifteenth day. Further characteristics of this organism are not given by Browne.

LACTOMYCES INFLANS CASEIGANA. Bochicchio

Bochicchio [1] isolated this species from Lombard cheese. It is a top yeast with unilateral budding. The cells are elongated, round or elliptical. When cultivated on gelatin, this yeast forms a whitish colony with an entire edge. It coagulates sterile milk and liquefies part of the coagulum without the formation of distinct amounts of acid. In lactose bouillon, between 25 and 40° C., it provokes an energetic fermentation. The most favorable temperature for this is situated at about 30° C. The temperature limit is about 60° C. Milk infected with this yeast is changed into a frothy mass with a disagreeable odor.

SACCHAROMYCES LEBENIS. Rist and Khoury

This yeast was isolated by Rist and Khoury [2] from leben where it is found with *Mycoderma lebenis*. It possesses oval cells (3 to 6 μ) with granular contents. The cells are isolated and one never finds mycelial formations. On sucrose agar below the surface, this yeast gives little growth and seems to grow only on the surface. However, one may, by successive culturings in this medium, cause it to take on anaerobic characteristics and the fermentation of sucrose. It grows well on ordinary gelatin without sugar and, at the end of 48 hours, forms white circular colonies with a moist and damp surface. Stabs into saccharose gelatin give colonies which are round and squeezed together not exceeding 3 to 4 μ. It does not liquefy the gelatin. On milk broth this yeast produces a cloudiness and later a sediment. It causes no fermentation. On grape must, it produces a cloudiness which is also followed by a deposit in the bottom of the culture flask. This species ferments saccharose and maltose but does not act on lactose. It works with *Mycoderma lebenis* in the alcoholic fermentation of lactose but it acts on this sugar only when associated with *Streptococcus lebenis* which probably decomposes the lactose.

[1] Bochicchio, N. Ueber innen Milchzucker vergärenden und Käseblähungen hervorrufenden neuen Hefenpilz. Cent. Bakt. 15, 1894.

[2] Rist, E., and Khoury, J. Études sur un lait fermente comestible, le "leben" d'Égypte. Ann. Past. Inst. 16, 1902.

TORULA KEPHIR. Nokolajewa [1]

This species was found along with many bacteria and *Torula ellipsoidea* in a decomposition of képhir. It is made up of round cells (3–4 μ in diameter); it develops with a red color on potato. This yeast ferments dextrose, saccharose and lactose.

TORULA ELLIPSOIDEA. Nikolajewa [2]

This yeast was found under the same conditions as the preceding. The cells are elliptical (6–9 μ in length and 3–4.5 μ in width) and develop on all substances. A yellow pigment is formed on potato. This yeast ferments dextrose and saccharose but not lactose

TORULA AMARA. Harrisson [3]

This yeast was isolated from a cheese and milk in America where it produced a bitter taste. Harrisson, who isolated it, showed that it came from cans of milk; the cans became infected from trees under which they were placed to dry. It produces a bad, disagreeable taste in milk at 37° C. after 14 hours. It produces an odor recalling that of plum stones. The taste is astringent. Later the milk coagulates and an aromatic ethereal odor is formed. The optimum temperature for budding is 37° C. and the temperature limits 48–50° C. This species easily ferments saccharose, dextrose and lactose. It grows in a bouillon containing 2.4 per cent of lactic acid.

DOMBROWSKI'S TORULA

Torula lactis α, Dombrowski: This yeast was isolated from Armenian mazun in Zürich by Düggeli and was described by Dombrowski.[4] The cells are usually oval; giant cells are often noticed in hanging drop preparations. On gelatin plates, the colonies are lenticular and are either circular or torpedo shaped. Growth in gelatin stabs extends only 4 cm. below the surface. The giant colony is flat and spread out with a slightly fringed border. In beer wort and must, this species acts like a top yeast. Fermentation is quite energetic.

[1] Nikolajewa, E. Die Microorganismen des Kefirs. Bull. Jard. Imp. St. Petersburg, 7, 1907.

[2] See reference for Torula képhir.

[3] Harrisson, F. C. Bitter milk and cheese. Cent. Bakt. 9, 1902.

[4] Dombrowshi, W. Sur l'Endomyces fibuliger. Comp. Rend. d. trav. du. labor. de Carlsberg. 7, Book 4, 1909.

The must is strongly discolored with the formation of an aroma. A scum is not formed, only a feeble ring. Clarification is generally bad. At the end of five and a half months in wort about 5 per cent of alcohol is formed. This yeast induces an active fermentation in wort with the formation of an aroma. It ferments dextrose, lactose, saccharose and d-galactose but has no action on maltose. Besides alcohol and carbon dioxide, it produces a small quantity of acid.

Torula lactis β, Dombrowski: This species was isolated by Burri and described by Dombrowski. It possesses cells of varied shapes. In solid media, they are generally elongated and united by a sort of mycelium. In wort cultures, they are spherical, elliptical, elongated or oval. The average dimensions are 7–9.5 μ in length and 4.25–4.5 μ in width. Giant cells are formed in hanging drops.

The colonies on gelatin or beer wort, in plates, are either torpedo shape or circular. They are made up of elongated cells resembling a mycelium. In stabs, development extends to about 4 cm. below the surface. The giant colony has a concavity in the center. Cultures on beer wort show a ring formation and a feeble attempt to form a scum. The wort is strongly discolored. There is the production of a slight aroma. After five and a half months, there are 6.3 grams of alcohol formed in 100 c.c. of medium. This species produces at 25° C. an active fermentation of milk with a slight disagreeable taste. It ferments lactose, saccharose, d-galactose and dextrose but does not act on maltose. Small quantities of acid are produced in the fermentation.

Torula lactis γ, Dombrowski: This species was found many times in kephir grains. It possesses oval cells, sometimes spherical, which have a diameter on beer wort of 3.5 μ, and which often possess numerous fat globules. Colonies on beer wort or gelatin plates are circular or shaped like torpedoes. In stab cultures growth extends about 4.5 cm. below the surface. Giant colonies possess a concavity in the center. This species produces a rather thick scum on beer wort which is of a whitish color and forms about 5 grams of alcohol in about five and a half months. It acts like a top yeast. It clarifies beer wort and produces an active fermentation. The wort is strongly discolorized with the formation of an aroma. At 23–25° C., this *Torula* causes an active fermentation in milk. It ferments lactose, saccharose, dextrose and d-galactose but does not act on maltose. Small amounts of acids are produced.

Torula lactis δ and *Torula lactis* ϵ, Dombrowski: These two species were encountered in various products from milk. They are only distinguished by the size of the cells. The cells are spherical in shape and often include a large fat globule. The cells of *Torula*

lactis δ are much smaller than those in *Torula lactis* ε. In the first they are 2.5–4.12 μ and in the other 3.1–5.6 μ. On gelatin in beer wort, in plates, both species form spherical or torpedo-shaped colonies. The giant colonies of *Torula lactis* δ have almost a flat surface while the others possess a concentrically folded surface. In beer wort or grape must, both species produce a fine ring but cause no fermentation. The wort is not discolored and there is no formation of an aroma. This does not ferment milk. Neither does it ferment lactose, saccharose, dextrose, d-galactose and maltose.

Torula No. 15, Dombrowski: The shape of the cells is oval. On plaster blocks, the cells possess a large fat globule. On beer wort gelatin plates, this species produces circular or torpedo-shaped colonies. The giant colonies show slight development with concentric zones. In carbohydrate liquid media, this species produces no fermentation but develops abundantly. At 23–25° C. the scum is a bright red. This yeast produces a strong cloudiness and a disagreeable odor. Many other milk yeasts have been isolated by Pierroton and Riboni Weigmann, Kalanthar, Jensen, and Mazé; they are too insufficiently known to be described here.

C. YEASTS FROM FATS

SACCHAROMYCES OLEI. Van Tieghem[1]

This yeast was accidentally observed by Van Tieghem in olive oil in which there were entrained droplets of water. It possesses oval cells arranged like heads in a chain. These bead-like structures break off and the isolated cells bud in order to form new ones. The cells measure on an average 4 μ and 2.5 μ. Their contents is a rose color. This yeast develops in all stretches of the medium without growing on the surface. The oil undergoes a marked change, becoming acid and saponifying.

ROGER'S TORULA

This yeast was isolated from different samples of butter by Rogers.[2] It possesses the property of decomposing fats with the formation of fatty acids. The cells are elliptical (3 to 5 μ) and show a slight tendency to form chains or masses. It ferments maltose slowly but does not act on other sugars (lactose, d-mannose, levulose, dextrose).

[1] Van Tieghem. Sur la vegetation dans l'huile. Bull. Soc. Bot. France. 28, 1881.

[2] Rogers, A. Eine gespaltende Torulahefe aus Buchsenbutter isoliert. Cent. Bakt. 10, 1903.

D. COLORED TORULA

Among the colored *Torula*, the red yeasts are the most numerous.[1] They are especially numerous in dust of the air. Some form mycodermic scums and may be classed as Mycoderma.

TORULA PULCHERRIMA. Lindner

Lindner[2] has found this *Torula* on numerous occasions, especially on various fruits and also on the excrement of potato bugs. Red pigments are formed.[3] In beer wort, its cells are at first elliptical but later they become larger and round with a large fat globule in their interior. They possess a thick membrane (Fig. 142). During germination the external membrane ruptures itself and an active budding takes place. This yeast ferments dextrose, d-mannose and levulose.

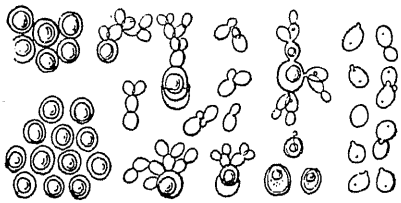


Fig. 142. — *Torula pulcherrima.* Old Cells and Their Germination (after Lindner).

TORULA MUCILAGINOSA. Jörgensen[4]

The cells are oval (5 to 5.6 μ long and 2 μ wide). Inoculated into beer wort, this yeast at first produces a slight cloudiness and at the same time a ring of a mucous yeast with a rose color as well as a mucous sediment visible only after shaking the culture. The ring continues to extend on the walls of the vessel which soon finds itself covered from top to bottom with a rose-colored growth. There seems to be no vegetation as a sediment. Clumps of this mucous ring may fall to the bottom of the flask. The surface colonies on gelatin with 1 per cent wort are round, faintly rose colored, moist, shiny and a little convex. The young colonies have a united border. The old ones are hollowed in the middle and provided with little transverse

[1] Beijerinck has described a *Saccharomyces pulcherrimus* which secretes a colorless chromogen which becomes a deep red in the presence of iron salts. He even suggests that this variety may be used as a test for iron. (Beijerinck, M. W. Chromogenic yeasts — a new biologic reaction for iron. Arch. neerland. physiol. 2 (1918), 609–15. Chem. Absts. 13 (1919), 1082.

[2] Lindner, P. Ueber rot und schwartz gefärbte Sprosspilze. Wochenschr. f. Brau. 4, 1887.

[3] All of the red yeasts have been grouped by bacteriologists into a special group known as "red yeasts."

[4] Jörgensen, A. Die Mikroorganismen der Gärungsindustrie. Berlin. 5th edition. Paul Parey. 1909.

furrows at the edges. This yeast produces no fermentation of dextrose, maltose, lactose, saccharose, raffinose and dextrine. It inverts saccharose and decomposes raffinose. In must with added alcohol, it forms, at the end of 8 days, a mucous ring if the alcohol does not exceed 2 per cent. With 5 per cent of alcohol, there is no development. The formation of a mucous ring seems to be related to the presence of albumin in the medium and concerned with the presence of carbohydrates. It increases when the amount of peptone added to the medium is increased.

TORULA CINNABARINA. Jörgensen [1]

This yeast, improperly designated under the name of *Torula*, seems to belong to the *Mycoderma*. The cells are oval or elongated often provided with short or long tubes, a sort of promycelium. Giant cells are often noticed either elongated or round. The long ones may be 14.6 μ in length and the round ones 9.5 μ in diameter. Cultivated in must or in solutions of the various sugars, this yeast produces a scum which, at first, is united, folded and of a red color. The liquid remains clear. No sediment is noticed at the bottom of the culture flask nor any fermentation. In old cultures, the wort undergoes a notable decoloration. At the end of 60 hours at 25° C., small islands of floating scum are produced in which a small number of cells begin to form a mycelium.



Fig. 143. — *Torula cinnabarina.* Scum on Old Culture (after Jörgensen).

At the end of 24 hours, the formation of a promycelium may become very abundant. On the promycelium and on the mother cells, the formation of buds may be seen. (Fig. 143.) The surface colonies on gelatin with 10 per cent of wort are round, with a faint red color. The old colonies are dry and show concavity and a finely fringed border. This yeast produces no fermentation in dextrose, maltose, lactose, saccharose, raffinose nor dextrine. It decomposed solutions of saccharose and raffinose. In wort with 1 to 2 per cent of alcohol added, there is a feeble development. If one decreases these amounts of alcohol, the yeast ceases to grow.

RED TORULA, NO. 36. Janssens and Mertens

This is a yeast a little smaller than *S. pastorianus* which in its scums seems to have a tendency to form elongated cells and filaments.

[1] Jörgensen, A. See reference for Torula mucilaginosa.

It was found in the bottom of a bottle of Maidstone beer and described by Janssens and Mertens.[1] On beer wort it develops on the surface and, after two days, produces a red scum. This develops very quickly and covers the whole surface of the liquid, later to become thick and folded. It also forms a reddish ring on the walls of the culture flask. The scum, if one shakes the culture flask, falls to the bottom and is replaced by a new one. If ammonium carbonate to 2 per cent is added there is no formation of this scum. The cells go to the bottom of the liquid and the solution becomes cloudy. Only when all of this ammonium carbonate has been destroyed does the scum form again.

On gelatin plates, this yeast produces surface colonies at the end of two days, visible to the naked eye. After 5 days, these colonies are entirely developed and possess a very characteristic appearance. There is a little enlargement in the middle and there is formed along their peripheries a slight fringe. This *Torula* liquefies gelatin very slowly. The optimum temperature for budding is situated between 20 and 25° C. Toward 30° C., the vitality of the yeast is somewhat diminished. This species produces no alcoholic fermentation and is not pathogenic.

The red pigment is almost insoluble in water and acetone but is quite soluble in carbon bisulfide. It seems to resemble carotine.

TORULA GLUTINIS. Pringsheim and Bilewsky

Syn.: CRYPTOCOCCUS GLUTINIS. Fresenius. SACCHAROMYCES GLUTINIS. Cohn

Fresenius[2] discovered this yeast which is very common in dust of the air. It is a common red yeast and has been since encountered by Cohn and Schröter.[3] Hansen[4] has also studied it under the name of *Cryptococcus glutinis*. He found that many species have been described under this name and that Cohn's yeast does not correspond with that described by Fresenius. Hansen has isolated many other species of red yeasts related to the *C. glutinis*



Fig. 144.—*S. glutinis* (after Hansen).

[1] Janssens, E. A., and Mertens, A. Étude microchemique et cytologique d'une Torula rose. La cellule, 20, 1903.

[2] Fresenius. Beiträge zur Mycologie. 1850.

[3] Cohn, F., and Schröter, J. Beiträge zur Biologie der Pflanzen. Vol. 1. 1872.

[4] Hansen, E. C. Saccharomyces colores en rouge et cellules rouge ressemblant a de Saccharomyces. Comp. Rend. des trav. lab. de Carlsberg. Vol. 1, Book, 24, 1879.

of Fresenius. One seems to correspond to the species described by Cohn and the other to a true *Saccharomyces* possessing ascospores and a third presents in beer wort budding cells like a true yeast but also develops a promycelium or germinating tube when it exists in a state of poor nutrition. (Fig. 145.)

Sartory[1] in 1907 reported a red yeast which he compared to the species of Fresenius. It is a yeast very widespread in nature and which one may find in macerations of grains, the rinds of certain cheeses and other organic substances. The cells are oval, the average dimension being 5 to 11 μ $\times$ 4 μ. The optimum temperature for budding is between 22° C. and 30° C. At 37 to 38° C., the yeast stops vegetating.

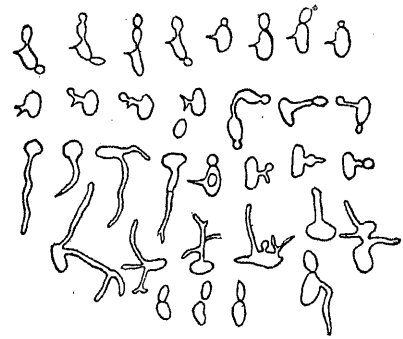


Fig. 145. — Rose Yeast Related to *S. glutinis* (after Hansen).

On glycerol broth, it forms a scum made up of cells which are associated to form a sort of mycelium. The sediment is made up of oval cells. On carrot, this yeast develops rapidly, giving a red layer. On plain potato, acid or glycerol, and on artichoke the development is less rapid. Small colonies are formed which have a reddish color. On gelatin and agar, the vegetation is less abundant and there is produced, after a certain time, a liquefaction of the gelatin. This yeast secretes invertase but produces no alcoholic fermentation. It is without action on maltose, d-galactose, starch and inuline. On milk, in about 14 days, there is a precipitation of the casein with no peptonization.

Quite recently, Pringsheim and Bilewsky have isolated another red yeast which is much like *Cryptococcus glutinis* of Fresenius which was named *Torula glutinis*. This yeast has no agreement with the yeast of Sartory.

The cells are spherical or oval (5 to 6 μ in length and 4 to 5 μ in width), isolated or united in budding formation but easily separable. They possess small granules and one or two large globules of fat. In culture, the cells possess a reddish color which may become brown under unfavorable conditions. The optimum temperature for budding is between 6° and 15° C. The minimum is about 0 and the maximum near 47° C. The cells, in the vicinity of the minimum and maximum temperature, are very small. Under certain conditions, giant

[1] Sartory, A. Étude biologique du Cryptococcus glutinis. Bull. de la myc. de France, 23, 1907.

cells with a diameter of 10–25 μ are formed along with long budding cells, incompletely formed and irregular. In liquid media the yeast forms a thin pellicle on the surface and a deposit in the bottom of the flask. On solid substrates, it forms small dots of growth about 0.5 to 1 μ in diameter. Later these run together forming a shiny mass almost mucous. The giant colonies on potato present a wrinkled appearance. On agar and gelatin, in streaks and stabs, the vegetation is at first with an even edge which after a certain time becomes furrowed. *Torula glutinis* does not possess a very characteristic appearance and a series of related yeasts have been described under this name.

CRYPTOCOCCUS BAINIERI. Sartory

This yeast was found by Bainier on the stems and leaves of the nettle where it lived as a saprophyte. It has been described by Sartory.[1] It is easily cultivated on all solid media (gelatin, agar, potato, both acid and glycerol), and especially on carrot. The colonies are of a beautiful deep rose color. On certain sugar media the color becomes a poppy colored red. The yeast gives abundant growth in liquid media (Raulin's solution, maltose, lactose, galactose or glycerine, Raulin's solution) and especially on glycerol bouillon. The optimum temperature for budding is situated between 24 and 26° C. The development begins at 15° C. and stops at 38° C. to 40° C. This yeast produces between 15° and 36° C. a rose-colored scum made up of elongated cells of larger dimensions than the cells in the sediment. It secretes invertase but does not ferment dextrose, maltose, lactose nor d-galactose.

PSEUDOSACCHAROMYCES STEVENSI. Anderson[2]

Anderson isolated this yeast from human feces and characterized it as follows:

"*Morphology.* In both young and old cultures the cells are narrowly elliptical, oblong or apiculate; cytoplasm, very granular; vacuoles, not distinct except in old, swollen cells; no elongated cells or false mycelium are formed under any condition of culture. Budding occurs only at ends, by elongation and swelling of the apiculate portion. The size is $2 \times 5\,\mu$. No endospores are formed.

"*Cultural Characters.* On glucose agar the streak is filiform, glisten-

[1] Sartory. Étude d'une levure nouvelle, le Cryptococcus Bainieri. Comp. Rend. Soc. de Biol. 61, 1906.

[2] Anderson, H. W. Yeast-like fungi of the human intestinal tract. Jour. Inf. Diseases, 21 (1917), 341–386.

ing, white, flat, and smooth. The growth is slow, and the colony becomes dirty-gray with age. In gelatin no liquefaction occurs; the growth is filiform. In beer wort and sugar mediums there is slow development, with no evidence of growth except a slight sediment. The giant colonies are very small.

"*Physiologic Properties.* There is no fermentation of glucose, levulose, sucrose, lactose, raffinose, galactose or maltose. No decided change in acidity occurs in these sugars, dextrin or yeast water. There is no change in litmus milk.

CRYPTOCOCCUS VERRUCOSUS. Anderson[1]

"*Morphology.* In young liquid culture the cells are oblong, narrowly elliptical or oblong-elongated; in old cultures elongated cells are common, with several 'oil' globules in each cell. The size is 3×9 microns. Budding occurs from shoulders, ends or sides. No endospores are formed.

"*Cultural Characters.* On glucose agar slant there is at first an even, filiform, glistening, white, smooth growth; later it becomes dull, brittle, verrucose and pulvinate. On carrot slant the growth is more profuse, with verrucose, and pulvinate. On carrot slant the growth is more profuse, with verrucose character more pronounced, and with chalky-white surface. There is a filiform or nodose growth in gelatin stab, with no liquefaction. On sugar mediums and beer wort, after 2 days, a few small, white patches appear on the surface, later becoming larger, dry and very firm; at first they remain separate, but later coalesce.

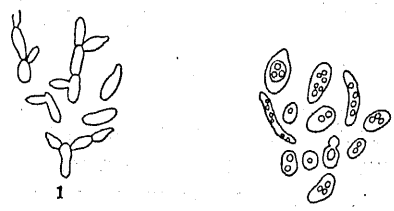


Fig. 145–A. — *Cryptococcus verrucosus*, Anderson.
1, Cells from Young Beer Wort Culture; 2, Old Cells.

"*Physiologic Properties.* It does not ferment glucose, levulose, sucrose, maltose, galactose, lactose or raffinose. No decided change in acidity occurs in these sugars. Litmus milk becomes very slightly alkaline after several weeks.

"The culture was isolated from human feces.

"The dry brittle character of the colonies on solid mediums, the formation of the isolated, white patches on all liquid mediums, and the peculiar type of cells, clearly distinguishes this yeast from any other studied."

[1] Anderson, H. W. Yeast-like fungi of the human intestinal tract. Jour. Infectious Diseases, 21 (1917), 341–386.

CRYPTOCOCCUS OVOIDEUS. Anderson

"*Morphology.* Cells in young cultures are round or oval, and fairly uniform in size and shape; in old culture cells are oval or broadly elliptical, varying markedly in size and with few budding cells. There are no elongated cells or hyphal elements. The size is $3.5 \times 4.5\,\mu$.

"*Cultural Characters.* On glucose agar the streak is filiform, slightly raised, glistening, smooth, and chalk-white. The growth is slow and there is little change in old cultures. There is a filiform growth in gelatin stab, with no liquefaction. No pellicle or ring is present in beer wort or in liquid sugar mediums.

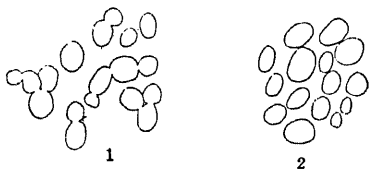


Fig. 145-B. — *Cryptococcus ovoideus*, Anderson.
1, Cells from Young Beer Wort Culture; 2, Cells from Old Glucose Agar Culture.

"*Physiologic Characters.* There is slight fermentation of glucose, levulose, and sucrose. This occurs only after a week and the production of gas is never over 10 per cent of the closed arm of the tube. No decided change in acidity occurs in sugar mediums. There is no change in litmus milk.

"The culture was isolated from human feces.

"This species is very similar in many of its characters to Culture 170.101. The latter, however, ferments glucose and levulose very rapidly and completely. Both of these cultures are slow growing, very smooth and remain white and even-edged in very old cultures. The surface elevation is not so decidedly convex as in most yeasts of the white, glistening type."

CRYPTOCOCCUS GLABRATUS. Anderson[1]

"*Morphology.* Cells in young cultures are oval or elliptical, and fairly uniform in size and shape; in old cultures cells are round, oval, or elliptical and more variable in form and size. Budding occurs from the ends or shoulders of the oval and elliptical cells. There are no elongated cells or hyphal elements. The size is $3 \times 4.5\,\mu$.

"*Cultural Characters.* On glucose agar the streak is filiform, glistening, raised, smooth, and chalk-white. In old cultures the surface remains smooth and the edge entire. There is a slow growth on all solid mediums; liquid mediums remain clear with little evidence of growth, and no pellicle or ring formation is present.

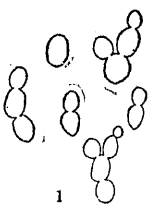


Fig. 145-C. — *Cryptococcus glabratus.*
1, Cells from Young Beer Wort Gelatin Culture.

[1] Anderson, H. W. See reference for Cryptococcus verrucosus.

"*Physiologic Characters.* There is rapid fermentation of glucose and levulose. Other sugars are not fermented. Litmus milk becomes only slightly alkaline. No decided change in acid reaction occurs in sugar mediums. Gelatin is not liquefied.

"The culture was isolated from human feces.

"This species differs in few respects from *Cryptococcus ovoideus.* The cells are more elliptical and the fermentation reactions are unlike.

CRYPTOCOCCUS AGREGATUS. Anderson [1]

"*Morphology.* In both young and old cultures the cells are mostly globular or slightly oval. No elongated cells are formed. Budding occurs from any point on the cell; usually several buds arise from each cell; in old cultures buds are commonly formed in large numbers about a single enlarged cell. The size is 3.5 μ.

"*Cultural Characters.* On glucose agar slant the growth is filiform, convex, glistening, smooth, chalk-white and firm. In old cultures the surface remains smooth, with even edges and no darkening in color. Filiform, later nodose, growth occurs in gelatin stab, with no liquefaction. No pellicle or ring is formed in beer wort or liquid sugar mediums. The surface of the giant colonies on glucose agar plates remains remarkably smooth, only dim, concentric lines appearing.

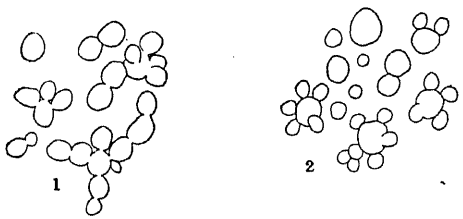


Fig. 145–D. — *Crytococcus agregatus,* Anderson.
1, Cells from Young Beer Wort Culture; 2, Cells from Old Culture.

"*Physiologic Properties.* There is no fermentation in glucose, sucrose, levulose, maltose, galactose, lactose or raffinose yeast water. No decided change in acidity occurs in these sugar mediums. Litmus milk becomes very slightly alkaline after 3 weeks.

"The culture was isolated from human feces.

"Two other cultures, isolated from the same person, were compared with the foregoing species and found to be identical. The isolations were made from the same sample of feces but from different colonies."

KRAMER'S RED TORULA

This species found by Kramer [2] in cider is a yeast which produces a top fermentation. It is provided with a red pigment soluble in water.

[1] Anderson, H. W. See reference for Cryptococcus verrucosus.

[2] Kramer, E. Ueber einen rotgefärbten bei Vergärung des Mostes Mitwirkenden Sprosspilz. Osterr. landw. Cent. 1, 1891.

It ferments dextrose and in a 10 per cent solution of this sugar produces 4.5 per cent of alcohol by volume. It inverts saccharose and ferments maltose. It has no action on lactose.

SACCHAROMYCES JAPONICUS. Yabe[1]

This yeast was isolated from some swampy fields in Japan on rice leaves. It is frequently encountered as is *S. keiskenna* in dust of the air in Japan. The cells are elliptical and slightly rounded. In Pasteur's medium, they measure $6 \times 3\,\mu$; in meat bouillon, $9.2 \times 5\,\mu$, sometimes reaching $10.3 \times 6.1\,\mu$. The budding is accomplished by a special method. The cells send out a long tubule about twice as long as the cell at the end of which there develops an enlargement constituting the bud. In certain cases, especially in peptone broth, this filament branches in place of budding and gives mycelial formations. This produces a red scum on liquids which falls to the bottom of the flask when disturbed. Stab cultures on carbohydrate gelatin after a few weeks show along the line of inoculation a feeble trace of growth. On the surface, on the contrary, a reddish pellicle is formed which develops progressively and liquefies the gelatin. This yeast is essentially aerobic producing no fermentation. The red pigment appears only in contact with air and is especially formed in cultures on potato. Saccharose and dextrose are good foods for this yeast, better than lactose. Alcohol to 3 per cent retards development; 7 per cent of alcohol prevents it. The cells die in 5 minutes at 45° C.

SACCHAROMYCES KEISKEANA. Yabe

This yeast was found by Yabe[2] along with the preceding one. Its cells are of a pale reddish color and are always spherical ($5.1\,\mu$ in diameter). Under good conditions of nutrition they may reach $9\,\mu$. The cells grow by a budding analogous to that of bottom beer yeasts. No mycelial formation exists. In stab cultures on gelatin, this yeast only produces along its line of inoculation a small number of cells which remain colorless; with the exception of those on the surface, no liquefaction is produced. The cells die in 5 minutes at 50° C.

[1] Yabe, K. On two new kinds of red yeast. Bull. of the Imp. University of Tokyo, 1902.

[2] Yabe. See reference for *S. japonicus*.

TORULA BOGORIENSIS RUBRA. De Kruyff

This is a yeast which was isolated from the soil of Java by Kruyff.[1] It possesses the very interesting property of fixing atmospheric nitrogen. It does not ferment any sugar, secretes amylase, lipase and sucrase and forms round colonies which have a reddish tinge in the center. Other rose-colored yeasts have been described as *Saccharomyces roseus* (Frank) Zopf and the *Torula roseaca* Van Hest.

TORULA RUBEFACIENS. Grosbusch[2]

The cells are round or elliptical (3.7–2–6 μ). There is abundant development in beer wort with great pigment production. This is red and soluble in water and exhales a fruity odor. Giant colonies on wort gelatin are strongly colored red. Gelatin is rapidly liquefied. On potato, the yeast gives a red colony. The production of pigment is influenced by the kind of sugar in which the yeast finds itself, fermentable sugars favoring this action. The concentration of the sugar and the amount of acid are also determining factors. The yeast ferments levulose and dextrose, acts less strongly on saccharose and a little on galactose. Ando,[3] in studying some red yeasts isolated from breweries which were probably *Torula*, found that the color did not depend upon the nutrient medium. The red pigment was found to have intimate connection with the life of the yeasts. In this case it was regarded as an indication of life.

Genus II. Pseudosaccharomyces. Klöcker

HANSENIA. Zikes

The cells are usually supplied at one or both ends with little points like those on lemons.

PSEUDOSACCHAROMYCES APICULATUS. Klöcker

Syn.: SACCHAROMYCES APICULATUS. Reess. Hansen

It has been stated that the yeast under the name of *Saccharomyces apiculatus* and described by Rees and Hansen represents not a species

[1] De Kruyff, E. Torula Bogoriensis rubra. Ann. Jard. bot. Buitenzorg. 3, 1909.

[2] Grosbusch, T. Ueber eine farblose, stark roten Farbstoff erzeugende Torula. Cent. Bakt. 42 (1915), 625–638.

[3] Ando, K. On red yeasts. Original Communications Eighth International Congress of Applied Chemistry, 14 (1912), 7–12.

but a group. Those which form spores are classed as *Saccharomycetes* and those which form no ascospores are called *Pseudosaccharomycetes.*

Saccharomyces apiculatus described by Reess and Hansen is a top yeast which causes active fermentation in dextrose but does not take it very far. After three months, according to Hansen, only 3 per cent of alcohol is formed. In beer wort, only 1 per cent of alcohol is formed. There is no fermentation of maltose nor inversion of saccharose.

Klöcker found this species in garden soil at Carlsberg. On wort at 25° C., it has lemon or ellipsoideus shaped cells (5–10 μ long). The temperature limits for growth are 36–37° C. and 0.5–3.5° C. It ferments dextrose, levulose, d-mannose and liquefies gelatin.

PSEUDOSACCHAROMYCES APICULATUS PARASITICUS. Klöcker

SACCHAROMYCES APICULATUS PARASITICUS. Lindner

Lindner discovered this yeast in 1895 in the body of an Homoptera *Aspidiotus Nerii* and also on the laurel, ivy, myrtle, etc. (Fig. 145-E.) It is probably from these plants that it gets into the bodies of insects. This species has the identical characteristics of *Saccharomyces apiculatus.* No formation of ascospores has been noticed. *Saccharomyces apiculatus parasiticus* is transmitted by the eggs and finally enters the larvae to penetrate their uttermost extremities. They do not seem to play a pathogenic rôle in *Aspidiotus* and seem to live in a sort of symbiosis. This yeast has not been cultivated. Hartig has found an apiculate yeast in the blood of caterpillars which is identical with that described by Lindner.[1] However, it differs in that it causes a fatal disease among caterpillars. Lindner believes that this yeast gets into the caterpillars from ivy which is abundant in the vicinity of Hartig's laboratory.

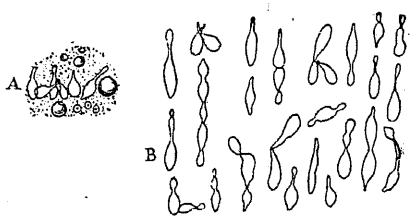


Fig. 145-E. — *S. apiculatus parasiticus.*

A, Cells Enclosed in the Protoplasm of the *Aspidiotus Nerii* cells; *B*, Greatly magnified cells (after Lindner).

[1] Lindner, P. Ueber eine in Aspidiotus Nerii parasitisch lebende Apiculatus Hefe. Cent. Bakt. 1, 1895.

SACCHAROMYCES MACROPSIDIS LANIONIS. Sulc [1]

This species was found in the pseudovitellius of certain *Lecanides* (*Macropsis Lanio*). They possess cells 3 μ in length and 1 μ in width. One of the extremities is pointed. (Fig. 129.) The contents show a nucleus and an alveolar protoplasm in the alveoli in which metachromatic granules are found. Multiplication is accomplished always at the poles. The buds are elliptical and of the shape of an egg. They separate from the mother cell, attain their complete development, and are never observed in chains. The *Saccharomyces macropsidis Lanionis* is closely related to, if not identical with, the *Saccharomyces apiculatus parasiticus*. It has not been cultivated.



Fig. 145-F. — *S. Macropsidis Lanionis* (after K. Sulc).

PSEUDOSACCHAROMYCES AUSTRICUS. Klöcker

On must at 25° C., the cells are ellipsoidal and 4 to 5 μ long. The temperature limits for growth are 35–36° C. and 0.5–3.5° C. It ferments dextrose, levulose and d-mannose. Gelatin is liquefied. It was found in soil from the Austrian Alps.

PSEUDOSACCHAROMYCES AFRICANUS. Klöcker

On beer wort at 25° C., the cells are elongated or lemon shaped (7–12 microns in length). The minimum temperature limits for growth are 36–37° C. It ferments dextrose, levulose, d-mannose and maltose very feebly. It was found in soil from Algeria.

PSEUDOSACCHAROMYCES CORTICI. Klöcker

This yeast has lemon-shaped cells on beer wort at 25° C. (6–11 μ in length). The temperature limits for growth are 36–37° C. and 0.5–3.5° C. It ferments dextrose, levulose, d-mannose and maltose very feebly. Gelatin is liquefied. It was secured from various trees about Copenhagen.

PSEUDOSACCHAROMYCES MULLERI. Klöcker

On beer wort at 25° C. the cells are small and shaped like lemons or ellipsoidal (4–6 μ in length). The temperature limits for growth are 35–36° C. and 0.5–3.5° C. It ferments dextrose, levulose and d-mannose and liquefies gelatin. It was found in soil from Java.

[1] Sulc, K. Pseudovitellius und ähnliche Gewerbe der Homopteren sind wohnstätten symbiötischer Saccharomyceten. Sitzungsberichte der König. Böhm. Gesellsch. der Wissenschaften in Prag. March 30, 1910.

PSEUDOSACCHAROMYCES LINDNERI. Klöcker

On beer wort at 25° C., the cells are small and either lemon shaped or ellipsoidal. The temperature limits for growth are 36–37° C. and 6–8° C. It ferments dextrose, levulose and d-mannose. It was found in soil from Java.

PSEUDOSACCHAROMYCES GERMANII. Klöcker

On beer wort at 25° C., the cells are lemon shaped (5–8 μ long). The temperature limits for growth are 36–37° C. and 6–8° C. It ferments dextrose, levulose and d-mannose and liquefies gelatin. It was found in soil.

PSEUDOSACCHAROMYCES JENSENII. Klöcker

On wort at 25° C., the cells are small and elliptical, resembling the shape of lemons (2–5 μ long). The temperature limits for growth are 5–6.3° C. and 37–38° C. It ferments dextrose, levulose, d-mannose, saccharose and maltose very feebly. Gelatin is liquefied. It was isolated from Java soil.

PSEUDOSACCHAROMYCES MALAIANUS. Klöcker

On gelatin at 25° C., the cells are shaped like lemons or sausages. The limits of temperature for growth are 36–37° C. and 0°–8° C. It ferments dextrose, levulose, d-mannose, saccharose, and maltose very feebly. Gelatin is not liquefied. It was isolated from soil from Java.

PSEUDOSACCHAROMYCES LAFARI. Klöcker

On beer wort at 25° C., the cells are elongated, in the shape of lemons or ellipsoidal. The temperature limits are 36–37° C. and 6–8° C. It ferments dextrose, levulose, d-mannose, saccharose and has feeble action on maltose. Gelatin is liquefied.

PSEUDOSACCHAROMYCES WILLII. Klöcker

On beer wort at 25° C., the cells are ellipsoidal or elongated and lemon shaped. They are small (4–10 μ in length). The temperature limits for growth are 37.5–38.5 and 6–8° C. It ferments dextrose, levulose, d-mannose, saccharose and maltose very feebly. Gelatin is liquefied. It was found in the soil of St. Thomas.

PSEUDOSACCHAROMYCES ANTILLARUM. Klöcker

On beer wort at 25° C., the cells are small and lemon-shaped or elliptical 5 to 12 μ long. The limits of temperature for growth are 37°–38° C. and 3–4° C. It ferments dextrose, levulose, d-mannose, saccharose and maltose feebly. Gelatin is liquefied. This yeast was isolated from soil from St. Thomas.

PSEUDOSACCHAROMYCES OCCIDENTALIS. Klöcker

On beer wort at 25° C., this species possesses lemon-shaped cells (6 to 10 μ long). The limits of temperature for growth are 39–40° C. and 3 to 6° C. It ferments dextrose, levulose, d-mannose and saccharose and acts feebly on maltose. It liquefies gelatin. It was isolated from soil from St. Croix.

PSEUDOSACCHAROMYCES SAUTRANZENSIS. Klöcker

The cells of this yeast are elliptical or lemon shaped on beer wort at 25° C. They are from 6 to 10 μ long. The temperature limits for growth are 37–38° C. and 3 to 6° C. It ferments dextrose, levulose, d-mannose and maltose very feebly. Gelatin is liquefied. It was isolated from soil from St. Croix.

PSEUDOSACCHAROMYCES INDICUS. Klöcker

On wort at 25° C., the cells are lemon shaped or elliptical. They may be sausage shaped (3–7 μ long). The temperature limits for growth are 37–38° C. and 3–4° C. It ferments dextrose, levulose, d-mannose, saccharose and maltose very feebly. It liquefies gelatin.

PSEUDOSACCHAROMYCES OF WILL

Will has isolated four species of yeasts from different sources none of which form ascospores. The shape of these yeasts is quite variable. The lemon-shaped cell with points may disappear and the cells assume the spherical shape. In other cases the cells may become spindle-shaped. Some of the cells are filiform while others are sausage shaped. The size of the cells is also quite variable. They vary between 5 and 6 μ in length. They may be distinguished from each other by their scums. Two of them (Nos. 4 and 7) have a well-developed scum while the other two (Nos. 1 and 3) form only a ring.

The giant colonies are characteristic in appearance; those for yeasts 1 and 3 spread out on the surface while those for yeasts 4 and 7 are cup-shaped. Yeasts 4 and 7 liquefy gelatin more quickly than the

other two yeasts and yeast 4 more quickly than 7. These four yeasts ferment dextrose and levulose. The fermentation continues for a long time with yeasts 4 and 7, longer than with the other two. The temperature limits for budding for these four yeasts are below 4° C. and 34–35° C. Yeasts 1 and 3 are more resistant to alcohol (ethyl) than the other two. Will considers yeasts 1 and 2 as two varieties of the same species which he designates under the name of *Pseudosaccharomyces cerevisiae* and yeasts 4 and 7 as varieties of another species to which he gives the name of *Pseudosaccharomyces vini*.

TORULA NIGRA. Marpmann [1]

Syn.: SACCHAROMYCES NIGER. Marpmann

This species was isolated from milk by Marpmann. It was regarded by this author as related to *P. membranaefaciens*. The cells are round or oval (1.5 to 3.0 μ in diameter). In sugar solutions, no mycelium is produced. On gelatin as in other substrates, black colonies are formed. This yeast does not seem to utilize saccharose and lactose but it uses a small quantity of dextrose. It seems to secrete either maltose, lactase, amylase, inulase, or invertase. Hansen [2] has shown that this yeast does not form ascospores and consequently does not resemble *P. membranaefaciens*. It is related to the genus *Dematium*. Guilliermond [3] has confirmed the opinion of Hansen and shown that this species possesses characteristics which class it with the *Dematium*. He has shown that on carrot it produces, at the end of 24 hours, a sticky mass composed of oval, slightly elongated cells, clothed with a sort of mucus which contains black particles. These are without doubt the black pigment seen in cultures. After a few days there is formed at the less moist parts of the carrot culture a very slender mycelium, which rises from the black mass of the yeasts. According to the investigations of Guilliermond the yeasts of this fungus include only a single nucleus and have a structure analogous to that of true yeasts, but the units of the mycelium may enclose many nuclei.

Hansen has observed two black *Torula* related to *Torula nigra*. Lindner has also described a black *Torula* cultured in Koch's labora-

[1] Marpmann, G. Cent. allgemeine Gewidlets Richard Landw. Jahrbucher, 1891.

[2] Hansen, E. C. Ueber rot und schwarzgefärbte Sprosspilze. Allg. Grauer- und Hopfenzeitung. 1887.

[3] Guilliermond, A. Recherches cytologiques sur les levures et quelques moisissures a forme levures. Thesis for the Doctorate at the Sorbonne. Rev. generale Botanique, 15, 1903.

tory which formed black yeast bodies and finally a deep green mycelium. This seemed to be related to Marpmann's yeast.

Other black yeasts have been mentioned by Marpmann under the name of *Schizosaccharomyces niger* and *Musa*. These are not well known and seem to be related to *Dematium* more than yeasts. They possess a complex mycelium. In all cases they have been improperly called *Schizosaccharomyces* for they are budding yeasts which possess none of the characteristics of the *Schizosaccharomyces*. There have been described may species which form a yellow and gray pigment. These are too insufficiently known to be mentioned here. *Saccharomyces sphoericus* might be mentioned. Browne has described a *Monilia nigra*, the characteristics of which are given later in this book.

In a recent investigation, Will isolated three forms of black yeasts which he regards as varieties of the same species. The three forms have a typical mycelium and a budding mycelium. The mycelium is a little branched and forms conidia which are ellipsoidal or spherical with thick walls. These multiply by budding, forming new yeasts or producing another mycelium.

In liquid media the three forms of yeast develop on the surface of liquid cultures and on the walls of the container with a typical mycelium. In the bottom of the flask there develops a flocculent sediment made up of yeast cells and mycelium. A ring develops around the side of the container and is cartilaginous, and a deep black in color. The scum is more or less colored a dark green; it is thick and quite tough. The giant colonies are a deep black. They are made up of a mycelium and budding cells. Growth for the three species stops at 35° C. The three varieties are killed in 30 minutes at 48° C. They do not develop in media with 4 per cent of alcohol added. They are slightly resistant to alcohol. No fermentations are induced.

Form I. The budding cells are ellipsoidal, elongated and sometimes apiculate (3.9–8.5 μ). Usually they are isolated but may be grouped, three or four cells being in a group. Some of the cells are giant cells.

Form II. The budding cells are oval, sometimes sausage-shaped (3.9–7.6 μ). They are sometimes grouped.

Form III. The budding cells are spherical, sometimes ellipsoidal or sausage-shaped. The mycelial structure is less developed than in the two preceding forms. Will found no relation between these yeasts and *Cladosporium herbarum*. On the other hand he does recognize relationships between these yeasts and *Dematium* but they are separated by other characteristics.

TORULA FROM "SOYA" MASH. Kita

Kita[1] examined different "soya" mashes and found a yeast which was much like *Saccharomyces soya*, Saito,[2] with the exception that no ascospores were found. Kita inoculated a sample of the mash into "soya" decoction containing salt. This was eventually plated out on "koji" gelatin to which 10 per cent of salt was added. Lindner's droplet method was finally employed for getting pure cultures.

The cells were usually round, sometimes elliptical with thick walls which were easily visible under the microscope. The plasma was wavy. Vacuoles were seldom seen. The size of the cells in "koji" decoction was 4.5–8 μ.

The colonies on "koji" extract-gelatin-agar were round or star-shaped, colored yellow, elevated in the middle with a smooth periphery. Giant colonies on the same medium are yellow, with a sunken center, granular surface and wavy periphery. Streak cultures are moist, yellow, granular and with wavy edges. In "koji" extract to which 10 per cent of salt has been added, growth is luxuriant. A ring is formed and the medium seems to contain suspended flocs. It ferments glucose, maltose, but not galactose, sucrose, lactose, raffinose nor arabinose. The optimum temperature for growth and fermentation is about 28° C. No endospores are formed by young cells on the plaster block. There seem to be no described species of *Torula* which agree with the characters of this one.

Genus III. Mycoderma.[3] Persoon

Under this name are grouped a number of yeasts which vegetate normally in contact with air and which form a scum but do not cause an alcoholic fermentation. At the beginning of the culture period, there is formed a folded sc m filled with air bubbles. Ordinarily long cells, budding at the ends with a transparent protoplasm with one or more refractive granules at both poles, are present. The *Mycoderma*, on the whole, seem to possess the characteristics of the fourth group of the *Saccharomycetes* (*Pichia* and *Willia*) and are perhaps asporogenic forms of the latter. Some of them are pigmented. The *Mycoderma* are very widespread in air and live especially on solutions containing alcohol.

[1] Kita, V. G. Hauptheфe der sojamaische. Orig. Communications 8th International Congress of Applied Chem. 14 (1912), 99–106.

[2] Saito, K. Cent. Bakt. Abt. 2, 17, 104, 152.

[3] It is well not to confound the *Mycoderma* with *Mycoderma aceti* which is a bacterium.

MYCODERMA CEREVISIAE. Desm, Hansen

Described by Hansen[1] after finding it in the breweries of Copenhagen this yeast possesses cells of varied shapes. Ordinarily the cells are transparent. Each cell usually contains one to three small refractive granules (Fig. 146). On beer wort, this yeast produces a dull gray scum frequently folded. It does not invert saccharose and gives no fermentation. On beer wort gelatin, spots of a gray color are formed. *Mycoderma cerevisiae* forms its sc ms between 2 and 15° C. and up to 33° C. It may cause considerable damage in beer which it attacks.

Hansen was the first to show that this yeast is not a well-defined species but rather a group of species which has been confirmed later by Lasché. This author describes four species which are distinguished from the yeast described by Hansen in that in beer wort, they produce alcohol, one 0.26 per cent by volume, two others 0.79 per cent and a third 0.51 per cent. All of these cause disease in beer. Lafar has discovered another *Mycoderma* very closely related to the latter which forms a scum quite closely related to that formed by *Mycoderma cerevisiae* and which gives acetic acid.

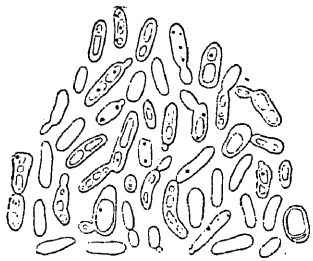


Fig. 146. — *Mycoderma cerevisiae* from a Brewery in Copenhagen (after Holm).

H. Leberle and Will have described two species of *Mycoderma cerevisiae*. The first *Mycoderma cerevisiae*, var. *a* has cylindrical cells sometimes elongated (2–3 μ wide and 7–10 μ long). The giant colonies are very uniform. The temperature limits for vegetative growth are: 7–30° and the optimum 20–25° C. This species assimilates only levulose. It oxidizes alcohol quite energetically and assimilates organic acids easily.

The second *Mycoderma cerevisiae* var. *c*, possesses oval cells or cylindrical cells (2–4 μ wide and 6 to 10 μ long). The temperature limits for growth are: 7° C. and 30° C. The optimum is 20–25° C. This species assimilated glucose and levulose; like variety *a* it acts towards alcohol and organic acids.

MYCODERMA VINI. Desm

This species has been described by Seynes, Wortmann and Winogradsky. It presents some of the characters of *Mycoderma cerevisiae*.

[1] Hansen, E. C. Levures alcooliques ressemblant a des Saccharomyces. Comp. Rend. des trav. du lab. de Carlsberg, 2, 1888.

The cells are oval and contain two vacuoles filled with refractive granules. At the beginning of their development the cells are united in budding chains. Later they separate. In old cultures, the yeast takes irregular shapes, some cells becoming angular. De Seynes thought that he saw ascospores in this species. This work was repeated by Engel, Reess and Cienkowski but not confirmed. It is then probable that these pretended ascospores were fat globules. *Mycoderma vini* is capable of changing the taste of wine. It contributes what is called the bouquet. It oxidizes alcohol, changing it into carbon dioxide and water with the production of acid. It does not attack tartaric very much and citric not at all, but destroys acetic acid and glycerol.

According to Siefert,[1] it is necessary to distinguish two types of *Mycoderma vini: Mycoderma vini I* and *Mycoderma vini II.* The first possesses cells 3 to 10 μ long and from 2 to 4 μ wide. The scum is at first smooth, later folded and grayish in color. The temperature limits for budding in wine with 8 per cent of alcohol added, are minimum, 5–6° C., optimum, 25–20° C. and maximum, 30° C. This species requires alcohol for development and attacks malic acid. In solutions containing 4.8 per cent of alcohol and malic acid, 1.52 per cent of glycerol is formed in 14 weeks. All of the alcohol is destroyed. In Austrian wine, in 26 days the amount of glycerol changes from 6.8 per cent to 82. It forms 9.04 per cent of acetic acid and the amount of alcohol changes from 7.8 to 3.8 per cent.

Mycoderma vini II has temperature limits lower that those for the above yeast: minimum, 1 to 2° C., optimum, 22° C. and maximum 28° C. to 30° C. It attacks malic acid only feebly. In Pasteur's solution in a week it gives 0.16 per cent of glycerol. The amount of alcohol is 4.8 to 4.1 per cent by volume. In Austrian white wine, after 26 days no increase in the amount of glycerol is accomplished. There is formed, however, 0.64 per cent of acetic acid. The quantity of alcohol decreases from 7.8 to 6.8 per cent by volume.

In a recent investigation, Gino de Rossi has shown that the species *Mycoderma vini* is really made up of a series of distinct varieties. By isolating the mycodermic forms from grape must or wine, which had been exposed to the air, this author has been able to characterize the species.

Mycoderma vini. On grape must, the cells are variable in form, oval, or elongated cylinders (5.6–9.5 × 2.8–4.8 μ), united in small groups which branch, but which separate in from 4 to 8 days into large cells with from 2 to 3 refractive granules. On gelatin with 10

[1] Siefert. Saccharomyces membranaefaciens. Ber. chem. physiol. Versuchsst. Klosterneuburg, 6, 1899–1900.

per cent of grape must, the colonies are white and round with a plain border. On wine or grape must, there is a scum in the beginning. The yeast gives no fermentation in must. It forms alcohol from wine without noticeably diminishing the acidity. The temperature limits are 2°–5° C. and 39° C., the optimum being 32°–35° C. Wine is sterilized by heating for 10 hours at 50° C. and 1 hour at 55° C. Direct sunlight in June produced the same results in 10 hours.

Mycoderma duplex. On grape must or wine, the cells are oval or pear shaped (3–7.2 × 2–3.6 μ). After 4 to 8 days, the cells are oval, small, and apiculate with either 1 or 2 refractive granules. Sometimes large oval or globular cells appear (5.4 × 10.2 μ).

On gelatin with grape must, the colonies are round with an entire edge. On grape must or wine, a white delicate scum is formed at the beginning adhering to the sides of the container. Finally, it breaks away and falls to the bottom as a fine deposit.

There is no fermentation in must, a slight diminution in the amount of alcohol in wine and a modification of the acidity. It is able to withstand 10 per cent of alcohol and 2 per cent of tartaric acid. The temperature limits are 5–7° C. and 39–40° C., the optimum being 35° C.

Wine containing this yeast is sterilized by 10 hours' heating at 48° C. and 1 hour's heating at 55° C. An exposure of 8 hours to sunlight also destroys it.

Mycoderma tenax. On grape must or wine, the cells are elliptical (4.8–8 × 2.8–3.8 μ) and solidly united in groups which branch. After 3–8 days, the cells are round, or oval, with a large refractive granule. On gelatin or grape must, the colonies are white and round with a plumose edge. On grape must or wine, a delicate scum is formed which clings to the walls of the culture flask but later falls to the bottom of the container. There is no fermentation in must, but a diminution of the alcohol and acid content of wine. It develops in the presence of 4 or 5 per cent of alcohol and 2 or 3 per cent of tartaric acid. The temperature limits are 12° and 32–35° C., the optimum being 30–32° C. Wine containing this yeast is sterilized by heating for 10 hours at 48° C. or 1 hour at 53° C. Exposure to direct sunlight for 10 hours will kill the yeast.

MYCODERMA HENNEBERG

Henneberg [1] mentioned two species of *Mycoderma* which he found in brewery yeasts and compressed yeast. These two species differ in

[1] Henneberg, W. Zwei Kahmhefearten an abgepresster Brennereihefe. Zeit. Ges. Brau. 26, 1903.

the shape of their cells. One of them produces filaments resembling the mycelium of *Monilia*. Finally they may be distinguished by their macroscopic appearance in solid media (giant colonies, streak cultures, etc.). In solutions of dextrose and levulose, both species form a scum which is filled with bubbles of carbon dioxide, the cells fall to the bottom of the culture flask and induce a very active fermentation. Both species, like *Willia anomala*, form ethyl ether. The optimum temperature for budding in these species is 32–41° C. These yeasts easily ferment dextrose and levulose but scarcely act on maltose and dextrine, and not at all on lactose, saccharose, raffinose, and inuline. In dextrose solutions, about 37 per cent of alcohol is formed by volume. Both species are able to utilize lactic acid as a food; they endure up to 5 per cent of this acid. They are also able to withstand quite large amounts of alcohol (11 per cent). The alcohol is rather rapidly oxidized to CO_2 and H_2O.

MYCODERMA CUCUMERINA. Aderhold

Discovered by Aderhold,[1] this species lives in beer and wine and brings about certain undesirable changes with an acrid taste. It oxidizes alcohol and lactic acid and produces from them volatile acids; however, it does not grow in more than 1 per cent of alcohol. This species may also transform alcohol into succinic acid, malic acid and tartaric acid.

MYCODERMA VALIDA. Leberle-Will [2]

The cells are cylindrical or oval (6–8 μ long and 2–4 μ wide). The temperature limits for growth are 1–45° C., optimum 20–25° C. This yeast assimilates dextrose and levulose and oxidizes ethyl alcohol very energetically. It assimilates the organic acids very easily, especially lactic acid.

MYCODERMA GALLICA. Lerberle-Will [3]

The cells of this yeast are either oval or cylindrical (7–10 μ long and 2–3 μ wide). The temperature limits for growth are 7 and 30° C. The optimum is 20–25° C. This species assimilates dextrose and levulose. It oxidizes alcohol quite energetically and easily assimilates the organic acids.

[1] Aderhold, R. Arbeiten der Botan. Abteilung d. Versuchsstation. d. pomolog. Inst. zu Proskau. Cent. Bakt. 5, 1899.

[2] Will, H. Beiträge zur Kenntniss der Gattung Mycoderma nach Untersuchungen von Hans Leberle. Cent. Bakt. 28, 1910.

[3] Will, H. See reference under Mycoderma valida.

MYCODERMA DECOLORANS. Will [1]

This yeast possesses cylindrical cells sometimes a little conical with a median constriction more or less marked. The dimensions are variable. The temperature limits for growth are 5 and 42° C. The optimum is 25–31° C. This species oxidizes alcohol very energetically. The giant colonies on must gelatin are very flat, thin and quite spread out. The edge is often lobate. The center is a little concave with the peripheral portion lined with concentric bands. This species causes a disease in beer characterized by a decoloration of the substrate, an odor and a musty taste.

SAITO'S MYCODERMA

Saito [2] has described four species of yeasts as *Mycoderma.* One isolated from "Shiro-koji" forms a dry scum folded, white and thick. The young cells are oval (4 to 6 μ) with abundant protoplasm, with one or more vacuoles and one or three fat globules. This yeast causes no fermentation.

The other two have been encountered in "Chinese yeast" from Corea along with *Saccharomyces Coreanus.* On sugar solutions, one forms a dry, thin, dull scum. The cells are ellipsoidal, often shaped like a sausage (4–8 μ long and 4–6 μ wide) with homogeneous contents and provided with small granules. It produces only a slow liquefaction of gelatin and a very feeble fermentation. The other, on the surface of sugar solutions, forms a farinaceous, white scum. The cells are oval or spherical (2–6 μ in diameter) and possess an interior with one or more fat globules. On gelatin streaks, the growth is snow white and presents a rough surface. Liquefaction is quite rapid. This species produces a feeble fermentation.

The fourth species was isolated from fermentation products of the soy bean. It has irregularly shaped cells, elongated or oval, much like those of *Saccharomyces pastorianus,* with a large vacuole containing refractive granules. On gelatin plates, this yeast produces small colonies with a moist appearance and with a slightly raised center. The edge is provided with fine indentations. It does not liquefy gelatin. On streak cultures, a grayish white deposit is produced and a finely indented border without liquefaction of the medium. The giant colony has a white appearance, the surface being much folded and irregular.

[1] Will, H. See reference under Mycoderma valida.

[2] Saito, K. Mikrobiol. Studien über die Soya-Bereitung. Cent. Bakt. 17, 1906; Notes on Formosan Fermentation Organisms. The Botanical Magazine, 15, 1902; Preliminary Notes on Some Fermentation Organisms of Corea. The Botanical Magazine, Tokyo, 23, 1909.

On decoctions of "koji," this species forms a thin scum smooth, shiny and dry. This is folded and in old cultures becomes farinaceous. On beer wort, the scum forms slowly and has a dull aspect. This yeast produces no fermentation of dextrose, levulose, d-galactose, lactose, maltose, saccharose, melibiose, mannose and raffinose.

BRUSENDORF'S MYCODERMA

Isolated by Brusendorf [1] from potatoes from the Danish Antilles, this species forms on hop wort a thick resistant scum with a dry appearance. The cells are oval, often slightly elongated and placed in chains of three or four individuals. They are 5 to 10 μ long and 2 to 5 μ wide. The cultures often have an acid odor due to formic acid produced by the yeast.

SACCHAROMYCES MYCODERMA I. Wehmer [2]

This yeast was isolated from fermenting sourkraut along with *Saccharomyces brassicae I* and *II*. The cells are always small (3.6 to 5 μ) with almost always a refractive granule of variable size. They provoke no fermentation. On cabbage decoction the scum is white, folded and tenacious. On gelatin, with cabbage decoction added, this yeast forms a fine sediment, white in color. This species destroys lactic acid energetically.

SACCHAROMYCES MYCODERMA II. Wehmer

Wehmer [3] isolated this species from the same source as the preceding one. It has ellipsoidal cells, never spherical but rather large ($8.4 - 4.8 \times 6\,\mu$). No fermentation is induced. On cabbage decoction the scum is thin and a dull gray. In old cultures, it becomes folded. This species quickly destroys lactic acid.

DUCLAUX'S YEAST. (Mycolevure)

This yeast was discovered by DuClaux [4] in Raulin's solution exposed to the air, where it appeared spontaneously. It develops with a regular scum which is folded when it lacks space to spread out. Under such conditions, it becomes very thick. The scum is formed of oval cells more or less granular. They are sometimes as large as

[1] Brusendorf. Ein Ameinsäure bildende Mycoderma. Cent. Bakt. 23, 1909.
[2] Wehmer, C. Untersuch. über Sauerkrautgärung. Cent. Bakt. 14, 1905.
[3] Wehmer, C. See reference for Saccharomyces mycoderma I.
[4] DuClaux, E. Traité de Microbiologie. Fermentation alcoolique. Vol. 3. Masson and Co., Paris, 1900.

ordinary yeasts but usually smaller. The cells are rarely united one to the other and are only grouped two by two. This yeast is a strong oxidizer. It oxidizes sugar to carbon dioxide and water. When introduced into a flask of sugar media which are easily aerated, the alcoholic fermentation is set up, but not as much sugar is transformed as by ordinary yeasts. It does not form more than 3 per cent alcohol.

MYCODERMA FROM PINEAPPLE. Kayser [1]

This yeast, isolated from pineapples, has elongated or elliptical cells (3.5–7 × 2.5–5 μ). Sometimes the cells are spherical, remaining attached in chains of 4 or 5 cells each. After 24 hours, in all carbohydrate media slight acid with a scum and ring is produced. The cells formed are like those formed in the deposit. In all media in which they grow, a pleasant ether odor is produced. The thermal death point in the moist state is around 53–55° C. and in the dry state 100–105° C. At these temperatures, the cells are killed in 5 minutes. Kayser has also isolated many mycoderma yeasts from bananas.

MYCODERMA LEBENIS. Rist and Khoury [2]

This species was isolated from leben. It has cells about 6–8 μ long and 3 μ wide, either isolated or forming groups in mycelium. In this latter case, the units are long and thin (33 μ long and 1.5 to 2 μ thick). The ends are enlarged, giving somewhat the appearance of biscuits. The lateral buds give rise to secondary chains at almost right angles. The protoplasm is finely granular with large fat globules.

On the surface of plain gelatin, the colonies are grayish white, opaque and a little raised, with a circular edge later indented with stratification in concentric zones. On carbohydrate gelatin, the colonies are exclusively aerobic and of a greenish gray color. The center is surrounded by an arborescent structure. Stabs in lactose gelatin develop abundantly on the surface but slowly in the depths. The culture resembles an inverted cone. On the surface of gelatin, a thin crust, dry, nacreous, much firmer in the periphery than in the center, is formed. No liquefaction of the gelatin is accomplished. In milk bouillon, the *Mycoderma* grows badly and forms a thin scum, transparent and gray, which is attached to the walls of the culture flask. The liquid becomes cloudy and there is a deposit in the bottom of the flask. There is no fermentation of lactose. On grape must, there is produced an active fermentation and a thick scum.

[1] Kayser, E. Note sur les ferments de l'ananas. Ann. Past. Inst. 5, 1891.

[2] Rist, E., and Khoury, J. Études sur un lait fermenté comestible, le "leben" d'Egypte. Ann. Past. Inst. 16, 1902.

This species ferments maltose, but has no action on saccharose or lactose. It seems to coöperate with *Torula lebenis* in the fermentation of milk but only when it is associated with *Streptococcus lebenis*.

DOMBROWSKI'S[1] MYCODERMA FROM MILK

Mycoderma lactis α. This yeast was encountered by Jensen and by Collau in various milk products, particularly in butter from Finland. The cells are elongated, rectangular, with rounded angles; sometimes they are slightly curved. Besides these, one may find numerous spherical cells. The cells enclose small droplets of fat. The dimensions of the cells are quite variable. After 96 hours on beer wort, the length may be 14.72, 13.0, 9.5, 8.42, 5.5 μ and their width, 4.15, 14.15, 3.7, 3.7, 3.2 μ. Often the cells may be longer than 27 μ. At the end of 24 hours, this species forms on carbohydrate liquid media, a well-developed scum. The wort becomes very cloudy and clears itself after 10 days. In must fermentation is brought about with the escape of an aromatic odor like that of ethyl ether. After five and one half months, 6 grams of alcohol are formed per 100 c.c. of must. In milk at 23–25° C., there is no fermentation.

On beer wort gelatin plates, the colonies are flat with a farinaceous covering in the midst. In gelatin stabs, development extends down to 3.5 ccm. About the line of inoculation, one may see extended lines which decrease in length as one goes toward the bottom of the tube.

Giant colonies have a membranous aspect with a grayish white color. In the center, a crateriform concavity exists about which is a raised portion. The border is finely fringed and possesses light folds. This yeast ferments only dextrose. The fermentation is accompanied with the formation of ethers.

Mycoderma lactis β. Collau isolated this species in Copenhagen from a culture of starter used in cream ripening. It is closely related to the Mycoderma described above but is distinguished by the size of its cells and by its fermenting ability. The appearance of the giant colonies is also a distinguishing characteristic. The cells may reach 12.87 μ in length and 3 μ in width.

On beer wort, this yeast acts like the preceding one; however, it has a very feeble fermenting ability. At the end of five months, only 4.2 grams of alcohol per 100 c.c. are formed.

The colonies on gelatin plates are much cut up and suggest the structure of molds. The cells are very much elongated, united and

[1] Dombrowski, W. Die Hefen in Milch und Milchprodukten. Cent. Bakt. 28, 1910.

possessing short lateral buds at their points of contact. This gives them the appearance of the mycelium of *Dematium*. Giant colonies show less development than those of *Mycoderma lactis* α. They are membranous and possess a concavity surrounded by an elevated portion. The edge is lightly folded and indented. The colony possesses a superficial crust of a whitish color.

Other Mycoderma have been mentioned but they are less known. Among them may be mentioned *Mycoderma sphaerpmyces* (Rothenbach) which ferments dextrine and *Mycoderma saprogenes saké* (Takahashi) which was found in an alteration of saké.

MYCODERMA CHEVALIERI. Guilliermond

This species was found along with *Saccharomyces Linderii* in the fermentation of an alcoholic drink similar to English ginger beer. On beer wort at 25° C., it develops rapidly, forming a sedimental growth after 24 hours. A scum is also formed on the surface. The scum appears as little floating islands which soon become confluent to form a continuous scum which adheres to the sides of the container, forming a marked ring. This scum is very thin and of a grayish yellow color. It does not contain air bubbles. It is very delicate and falls to the bottom of the culture flask when it is disturbed. Another re-forms very quickly. It has the same characteristics as the scum formed by *Zygosaccharomyces Chevalieri*.

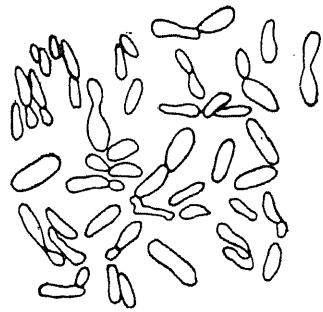


Fig. 146–A. — *Mycoderma Chevalieri*. Cells from Sedimental Growth after 44 Hours (after Guilliermond).

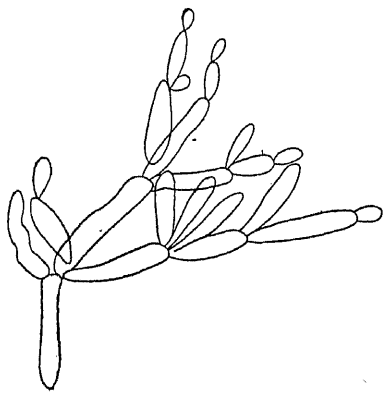


Fig. 146–B. — Mycelial Formation in *Mycoderma Chevalieri*, Showing Attached Yeast Structures (after Guilliermond).

When examined at the end of 24 hours, the sediment shows almost constantly yeasts isolated or united two by two. These are generally small and elongated; rarely are they short or oval. Their dimensions vary between 3 and 5 μ wide and 4 and 14 μ long. Their average size is about $3.96 \times 6.21\ \mu$. The contents of the cells are quite transparent with one or two large vacuoles less dis-

tinct with often many fat droplets. Budding is uniquely accomplished at both ends of the cells. The cells possess the characteristic appearance of *Mycoderma*.

The scum is, at first, almost always made up of yeast cells. These have somewhat the same appearance as the cells in the deposit. They are rarely isolated as in the sediment and are more often united in groups of from 4 to 8 cells. Certain cells have a tendency to elongate and may reach 14 to 20 μ in length.

After from 14 days to 2 months, a mycelial formation appears in the sediment. The temperature limits for growth are 5° C. and 46–57° C. On wort gelatin the giant colonies have a characteristic appearance. The center is a creamy yellow color and is made up of fine reticulations. The periphery is made up of two zones; first, one with a white color and thick, secondly, one with canals running out to the edge from this center. The gelatin is liquefied. On wort gelatin at 20° C., the colony is grayish white with a dry appearance. The yeast causes a slight fermentation in beer wort and ferments saccharose, feebly dextrose, energetically levulose and d-mannose.

MYCODERMA SP. Saito

This species forms on beer wort a white thick scum which adheres to the sides of the containers. The cells are oval and often curled. The giant colonies develop with a thick gray vegetation. The temperature limits for growth are 2–3° C. and 32–35° C. On "koji" decoction, this yeast gives no fermentations.

MYCODERMA OF FISCHER AND BREBECK

Fischer and Brebeck [1] have described a number of *Mycoderma* under the generic name of *Endoblastoderma* and *Blastoderma*. Such are *End. amycoides I–IV*, *liquefaciens*, and *glucomyces I–IV* and *Blastoderma salminicolor*. The last one is most interesting and the best known. It was found in a sample of sea water south of the island of Azores. The most salient characteristic of this species is that the cells form long extensions at the end of which develop structures like conidia. These quite often develop on the surface of the liquid in contact with air. When examined in a hanging drop, they possess an excessive brilliant aspect. This species possesses a red pigment.

Two other red Mycoderma have been described or rather observed by Lasché. *Mycoderma humuli*, isolated from hop leaves and *Mycoderma rubrum*, found in a culture of contaminated gelatin.

[1] Fischer, B., and Brebeck, C. Zur Morphologie, Biologie und Syst. der Kahmpilze, Jena, 1891.

MYCODERMA MONOSA. Anderson[1]

"*Morphology.* Cells in young cultures are elliptical or narrowly elliptical; in old cultures cells are of various forms, predominantly elliptical, with numerous elongated and irregular forms. Rows of elongated cells in old cultures form a false mycelial development. No true septation is observed. Budding occurs from the ends or from shoulders of the young cells. The size is $2 \times 5.5\,\mu$.

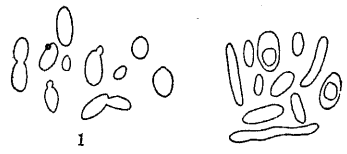


Fig. 146–C. — *Mycoderma monosa*, Anderson.
1, Cells from Young Beer Wort Culture; 2, Cells from Old Culture.

"*Cultural Characters.* On all agar slants the streak is spreading, dull, white, flat, and becoming gray with age. A heavy dull pellicle is formed within 24–48 hours on all liquid sugar mediums and on beer wort. There is a villous growth along stab in gelatin.

"*Physiologic Properties.* Glucose and levulose ferment readily. There is no change in litmus milk. Sugar mediums, with an original acidity of − 1, become less acid after 1 week. The culture was isolated from human feces."

MYCODERMA RUGOSA. Anderson

Anderson isolated this yeast from human feces and characterized it as follows:

"*Morphology.* Cells in young cultures are elliptical, oblong, elongated, or somewhat irregular; in old cultures the cells on the surface of the medium are oblong, ovate or elongated; beneath the surface very long, narrow cells of hyphal character are produced by the elongation of the bud at the distal end of another elongated cell. No septate mycelium is formed. Budding in young cells occurs from end or shoulder. The size is $3 \times 6.5\,\mu$.

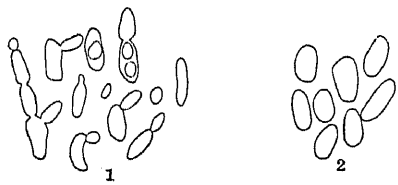


Fig. 146–D. — *Mycoderma rugosa*, Anderson.
1, Budding Cells from Young Beer Wort Culture; 2, Cells from Old Culture.

"*Cultural Characters.* On glucose agar slant the streak is white, dull, and flat, but not spreading; later the surface becomes glistening and decidedly rugose and pitted. Bushy growths may extend

[1] Anderson, H. W. Yeast-like fungi of the human intestinal tract. Jour. Infectious Diseases, 21 (1917), 341–386.

downward into the agar at points along the streak. There is a rapid villous development in gelatin stab cultures. A heavy pellicle is formed in sugar mediums and beer wort. Giant colonies are very distinctive.

"*Physiologic Characters.* No sugars are fermented; there is no change in litmus milk.

"This mycoderma is not distinguishable from several other species, for example, *M. cerevisiae,* as far as the morphologic and physiologic characters enumerated are concerned. An examination of photographs of the giant colonies of various *Mycoderma* species revealed the fact that none of these species produce the peculiar rugose-pitted type formed by the foregoing species. The production of such type of growth is not confined to giant colonies on glucose agar, but is present on slants of glucose and beer wort agar."

MYCODERMA TANNICA. Asai

Asai [1] has isolated a new yeast which causes dark brown or black spots on leather. The yeast grows in dextrose or levulose or other sugar solutions with an ammonium salt or amino acid as the source of nitrogen. It does not grow readily in dilute tannin solution but when dextrose and amino acids are added good growth takes place. Small amounts of alcohol and carbon dioxide are formed.

MYCODERMA ACIDIPANI. Rossi [2]

The cells are oval in shape (3.2–6.6 × 2.3–3 μ) and are united in branching groups. There are 1 or 2 refractive granules in each cell. On must gelatin, the cells are white, round, and provided with a slightly filamentous border. In grape must, or wine, the delicate scum is at first compact, later thick and adherent to the walls. If agitated, it falls to the bottom of the container. There is no fermentation in must, but a notable diminution in the content of alcohol and a marked increase in acidity. This yeast normally develops at a concentration of from 9 to 10 per cent of alcohol and withstands from 1 to 2 per cent of tartaric acid. The temperature limits are 2–5° C. and 32° C. The optimum is between 22° C. and 27° C. Wine containing this yeast may be sterilized by heating for 10 hours at 45° C. and 1 hour at 50° C. or by an exposure of one hour to direct sunlight.

[1] Asai, T. Physiological investigation of a new yeast which flourishes in tanning liquors. Jour. College Science Imperial University, Tokyo, 39, 1–42. Journal of the Chemical Society, January, 1919.

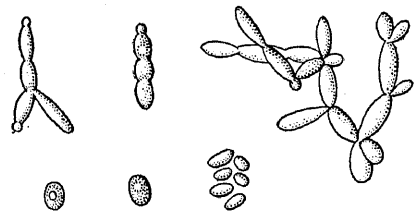
[1] Rossi, G. Micoderma del vino. Le Stazioni Sperimentali Agrarie Italiene. 50, 1917.

Genus IV. Medusomyces. Lindau

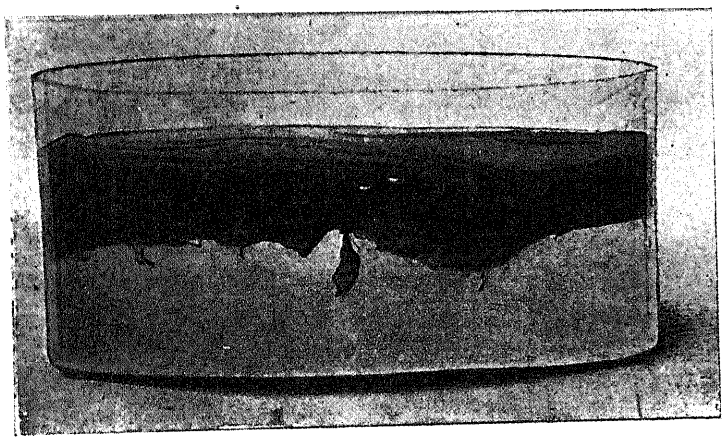
Mycodermic yeasts with a thick gelatinous stratified scum, resembling somewhat a medusa.

MEDUSOMYCES GISEVII. Lindau

This yeast was secured from Doctor Gisevii [1] from the region of Courland where it is used as a household remedy. It was carefully studied by Lindau. It is easily cultivated and macroscopically forms a peculiar covering on liquid media. Lindau found tea infusion the best liquid medium on which to propagate it. This covering does



1



2

Fig. 146–E. — *Medusomyces Gisevii.*

1. Yeast cells; 2. Scum developing in a Culture Flask.

not have the appearance of ordinary scums but is made up of an elastic tenacious mass. The liquid soon assumes an aromatic, fruity odor. In older cultures the covering becomes a brownish yellow color. Microscopic examination of this covering shows the presence of numerous round or elliptical cells. The length varies between 5.5–8.5 μ

[1] Lindau, G. Ueber Medusomyces Gisevii, eine neue Gattung und Art der Hefepilze. Berichte deutsch. Bot. Ges. 31 (1913), 243–248.

and the width between 1.5 and 3.8 μ. Budding takes place at the poles. (See Fig. 146–E.). The formation of the slimy substance about the cells is not thoroughly understood but is probably intimately connected with the outer cell wall. The peculiar characteristics of this yeast along with those of the scum caused Lindau to propose a special genus of *Medusomyces*. He separates this yeast from the *Mycoderma* by the characteristics of the scum.

Lindner [1] examined some of the material from Courland which was given to him by Lindau. He found different fungi among which was *Bacterium xylinum* to which he attributed the fermenting capacity of this material. The presence of different yeasts, such as *Saccharomyces Ludwigii* and *Schizosaccharomyces Pombe*, was also suggested.

[1] Lindner, P. Die vermeintliche neue Hefe Medusomyces Gisevii. Ber. deutsch. Bot. Gesell. 31 (1913), 364–368.

CHAPTER XII

PATHOGENIC YEASTS

THE pathogenic yeasts, which do not sporulate, possess generally the characteristics of *Torula*. They may be regarded as part of this genus. However, Vuillemin has created a special generic name for them, *Cryptococcus*. This name is generally used and so it has been retained for this discussion.

CRYPTOCOCCUS DEGENERANS. Vuillemin

Syn.: BLASTOMYCES VITRO SIMILE DEGENERANS. Roncali [1]

This yeast was encountered in a ganglion of the armpit of a woman attacked by a cancer and in other tumors. It was both extra- and intracellular. In cancer the cells were rounded, rarely oval, isolated or in groups, without capsules, with homogeneous contents, poor in granulations. In cultures, the cells are elliptical, mixed with mycelial forms. In carbohydrate solutions, this organism forms a scum composed of yeasts and mycelium. In bouillon, it produces an abundant sediment made up of cells and filaments. On gelatin plates, the superficial colonies are irregular, of a grayish yellow color; there is no liquefaction. On gelatin streaks, the growth is milky white. On potato, the colonies are grayish white and undulated. The yeast does not ferment saccharose. It is pathogenic for guinea pigs. Injections into the peritoneum cause death of the animal in 15 to 30 days.

CRYPTOCOCCUS GILCHRISTI. Vuillemin

Syn.: ZYMONEMA GILCHRISTI. De Beurmann and Gougerot. — BLASTOMYCES DERMATITIS. Gilchrist and Stokes

This yeast was found by Gilchrist [2] in a case of scrofular dermatitis and later by Gilchrist and Stokes in a case of pseudo lupus vulgaris. It has round, slightly oval cells, 20 or more μ in diameter,

[1] Roncali. Die Blastomyceten in den Adeno-Carcinoman des Ovariums. Cent. Bakt. 18, 1895.

[2] Gilchrist. A case of blastomycetic dermititis in man. Johns Hopkins Hospital Bulletin. 1896.

and a thick membrane. (Fig. 147.) In cultures, it has cells without capsules, elongated and mixed with mycelial filaments. No alcoholic fermentation is brought about nor scum formed on carbohydrate media. It does not liquefy gelatin. It is hardly pathogenic for animals.

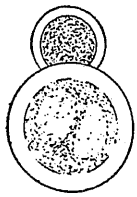


Fig. 147.—*Cryptococcus Gilchristi* (after Gilchrist).

Echon-Echeug have recently found a yeast in the serous secretion from a lesion in the cervical region, simulating cutaneous tuberculosis. The cells are spherical (7–16 μ), united two by two. The cultures on Sabourand's agar has yielded white colonies which become brownish, made up of a mycelium producing cells like those found in the lesions.

CRYPTOCOCCUS TOKISHIGEI (Tokishige). Vuillemin

Syn.: CRYPTOCOCCUS FARCIMINOSUS, Rivolta and Micellone. — SACCHAROMYCES EQUI, Marcone. — CRYPTOCOCCUS RIVOLTAE, Fermi and Aruch. — PARENDOMYCES OF RIVOLTA AND MICELLONE. Beurmann and Gougerot

This yeast was discovered by Rivolta and was considered by this author as the parasite of epizoötic lymphangitis or African glanders, a communicable infection of horses and mules. Numerous authors have thought that they cultivated this organism. Fermi and Aruch thought that they obtained it on potato and San Felice said that he reproduced the disease by cultures.

Marcone and Tokishige were the first to obtain the development of the fungus but they were unable to cultivate it in series. Tokishige in Japan has been able to obtain colonies on quite diverse media, but could not produce the disease when inoculating a horse with the colonies. More recent studies by Négre and Bride and Négre and Boquet have demonstrated that the parasite of this disease is indeed a yeast. In a few animals, the organism possessed the shape of a yeast. They secured best growth of the *Cryptococcus* by sowing a drop of pus on horse dung agar and covering it with the deposit of a maceration of lymphatic ganglions. The colonies are

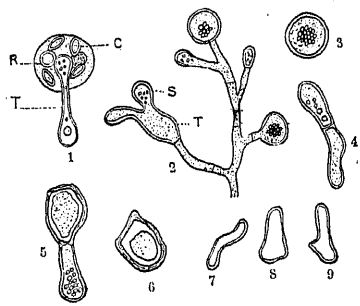


Fig. 148. — *Cryptococcus Tokishigei.*

1, Cryptotoccus in a leucocyte. R, Round Form of the Cryptococcus. T, Mycelial tube forming a bud. The bud is still within the Leucocyte. — 2, Mycelial Tubes Formed by the Budding of an External Spore S. T, Mycelial Tube. — 3, External Spore. — 4, Its Bud. — 5, Chlamydospore in a Mycelial Tube. — 6, Free Chlamydospore. — 7–9, Free units of an old culture.

then transplanted onto the same medium as Sabourand's medium. The organism is easily cultured and develops rapidly.

The most favorable temperature is 37° C. At this temperature the colonies on Sabourand's agar have a yellow sandy appearance. They are folded and have little white points.

In the beginning the *Cryptococcus* enlarges and assumes a round shape filled with oil droplets. It then buds giving mycelial tubes which form lateral branches.

On the secondary branches occur tertiary branches. At the end of all of the filaments small buds form, the walls of which thicken and the contents become filled with fat globules. These detach themselves and become external spores. These are probably the forms of the organism which multiply under the shape of yeasts. In culture, the spores form new mycelial structures. The mycelium is also able to form at the ends of the filaments a small number of segments with chlamydospores having a very thick wall and finely granular contents. In old cultures, the units of the mycelium break off.

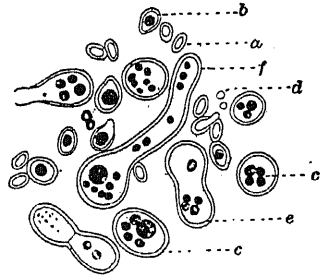


Fig. 148–A. — *Cryptococcus Tokishigei*, Cultivated on Nutritive Agar.

a, Typical cells; *b*, *c*, Cells Containing Granules Considered by Tokishige as Ascospores; *d*, Free Granules Supposed to have Sprung from the Cells; *e*, *f*, Transformation of the Cells into Filaments (after Tokishige).

At times the authors have noticed cells with three or four elements resembling ascospores. This would tend to make the yeast an *Endomyces*. The presence of ascospores does not seem to be well established. As to the ascospores described by Tokishige,[1] Negre and Boquet have shown that they were simply granular bodies.

Cultures of the yeast inoculated into a horse by scarification of the epidermis and subcutaneous injection produced abscesses and finally a clinical history of the natural disease. The *Cryptococcus* appears in the lesions three to four weeks after inoculation. The cells are at first isolated and have the shape of small oval units with thin walls. Later they take on a double contour and appear inside of the leucocytes. The serum from sick animals gives positive reactions with cultures of the fungus.

Boquet and Negre[2] have studied the variations taking place in the development of Rivolta's *Cryptococcus*. They found that a minimum temperature of 15–18° C. caused this parasite to take on a mycelian structure. At the optimum temperature, 35–36° C., in liquid

[1] Tokishige. Ueber pathenogene Blastomyceten. Cent. Bakt. 19, 1896.

[2] Boquet, A., and Negre, L. Polymorphisme morphogenique du Cryptococcus de Rivolta. Ann. Past. Inst. 33 (1919), 185.

media, the cells became round or oval and were surrounded by a double membrane. Whether the parasite reproduced by budding or not seemed to be independent of aerobiosis.

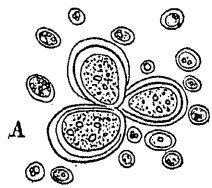
CRYPTOCOCCUS FARCIMINOSUS. Rivolta and Micellone

Syn.: SACCHAROMYCES EQUI. Marcone. — CRYPTOCOCCUS RIVOLTAE. Fermi and Aruch. — PARENDOMYCES DE RIVOLTA AND MICELLONE. De Beurmann and Gougerot



Fig. 149. — *Crypt. farciminosus.* Growth of Cells and Asci in the Pus (after Fermi and Aruch).

This species has been regarded as the causal organism of glanders which attacks horses and mules. It has round or oval cells, sometimes pointed at the poles, with granular contents (Fig. 149). It is easily cultivated in all media. On potato, it produces a round, raised colony with a dirty white color. It scarcely develops on agar. Fermi and Aruch [1] have described in the cells of this yeast found in pus, globules which they regarded as ascospores. These could not be found in artificial cultures, however.



CRYPTOCOCCUS HOMINIS (Busse). Vuillemin

Syn.: ATELOSACCHAROMYCES HOMINIS. De Beurmann and Gougerot

Discovered by Busse,[2] in chronic periostitis of the tibia, this yeast, *in situ*, possesses cells which are round or oval, unites in various numbers in a substance with a homogeneous appearance, making a sort of common capsule. (Fig. 150, B.) In cultures, the same shape is presented but there is no homogeneous substance. There is a membrane with a double layer which thickens as the culture becomes older. (Fig. 150, A.) It is cultivated easily on all media between 15° and 38° C. In liquid media a sediment is formed and on prune juice it finally forms a scum with a dirty gray color. In gelatin stabs, the colonies are white and

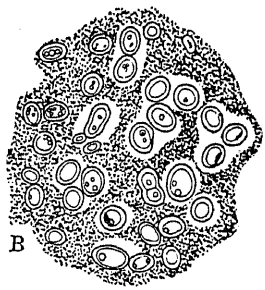


Fig. 150. — *Cryptoc. hominis.*

A. Old Culture on Prune Juice. — *B.* Numerous Yeast Cells in a Giant Cell (Abscess of the Human Shinbone) (after Busse).

[1] Fermi, C., and Aruch. Ueber eine neue pathogene Hefeart und über die Natur des sogennanten Cryptococcus farminosus, Rivolta. Cent. Bakt. 17, 1895.

[2] Busse, Ueber Saccharomyces hominis. Virchow's Archive, 40, 1895.

shiny but there is no liquefaction. On potato, the colonies soon unite to form a thick dirty white layer. This yeast ferments dextrose. It is pathogenic for rabbits, white rats and dogs.

CRYPTOCOCCUS LINGUAE-PILOSAE. Vuillemin

Syn. SACCHAROMYCES LINGUAE-PILOSAE. Raynard and Lucet

This yeast was discovered by Raynard and Lucet in a sickness called black tongue. Lucet who has studied this disease experimentally has shown that it may not be reproduced. According to Guegen [1] and Thaon,[2] this yeast acts only in association with *Oospora lingualis*. There seems to be a sort of symbiotic association between these two fungi. This yeast has round or oval cells, often elongated, in which the buds often remain united to the mother cell. This gives the appearance of a pseudo-mycelium. (Fig. 151.) On glucose, levulose, glycerol and especially potato decoctions, or fruit decoctions, after 10 hours at 37° C., there is good growth. Later the scum thickens and becomes gray or reddish. It may also become folded with a ring. On gelatin this yeast forms a mucous layer, white, shining, with contours. On potato it forms a thin layer, dry and brown. The optimum temperature for budding is found between 25 and 35°.

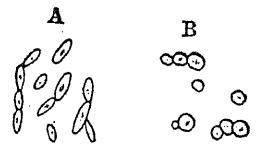


Fig. 151. — *Cryptococcus linguae-pilosae.*

A. Cells developed on Scum in Raulin's Liquid. — *B*. Cells Isolated from the Muscles of the Tongue.

This yeast ferments glucose and levulose. It is pathogenic for animals.

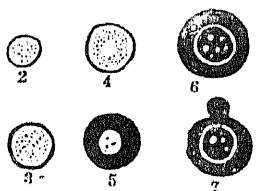


Fig. 152. — *Cryptococcus lithogenes.*

2 to 4. Young Cells. — 5 to 6. Cells which have Undergone a Chalky Degeneration (after San Felice).

CRYPTOCOCCUS LITHOGENES. Vuillemin

Syn.: SACCHAROMYCES LITHOGENES. San Felice

This yeast was discovered by San Felice [3] in the lymphatic ganglions of a cow which died from generalized carcinoma. In the animal it possesses round cells of variable forms and dimensions, sometimes enclosed in a calcified capsule, with brilliant granules in the protoplasm (Fig. 152).

[1] Guegen, F. Sur Oospora lingualis et Cryptococcus linguae-pilosae. Arch. de parasitol. 12, 1909.

[2] Thaon, P. Symbiose de levure et oospora dans un cas de langue noire. Soc. de Biol. 67, 1909.

[3] San Felice, F. Ueber eine für Thierpathogene Sprosspilzart und über die morph. Uebereinstimmung welche sie bei ihren Vorkommen in den Gesseln mit Krebsascidien zeigt. Centr. f. Bak., V. XVII. 1895.

In a culture one finds small cells with homogeneous color and with fine membranes intermingled with large cells containing a refractive body in their centers.

On glucose broth this species forms a heavy sediment and very often a scum. On gelatin plates the surface colonies are round like pinheads and the deep colonies smaller and of a yellow color. In gelatin stabs this species forms a white moist layer and numerous colonies. It does not produce liquefaction. On potato it gives a fine thick pellicle.

This yeast is pathogenic for guinea pigs and rabbits.

CRYPTOCOCCUS GRANULOMATOGENES. Vuillemin

Syn.: SACCHAROMYCES GRANULOMATOGENES. San Felice

Discovered by San Felice in nodules on the lungs of pigs, this *Cryptococcus* possesses round or slightly oval cells with a variable edge with contents either homogeneous or vacuolar, and with a bright central granule.

On glucose broth it produces a cloudiness very rapidly, and later a scum. On gelatin plates it gives round white colonies. The surface colonies are larger. On gelatin stabs it produces a white layer a little raised, accompanied along the line of inoculation by a train of small yellow colonies. No liquefaction is produced. On potato the culture is elevated and slightly grayish in color. This yeast produces a red pigment on honey and slices of pear. It is slightly pathogenic for animals.

CRYPTOCOCCUS NIGER (Maffuci and Sirleo). Vuillemin

This species was discovered by Maffuci and Sirleo[1] in a pulmonary myxoma from a guinea pig inoculated with the liver of an embryo coming from a tuberculous mother. In the myxoma and in cultures it possesses round or oval cells with a rather thick membrane and a protoplasm supplied with nuclear bodies. The cells remain attached two by two.

On liquid media the vegetation forms a white deposit and no scum; on gelatin streaks a whitish growth is secured; on gelatin stabs a whitish growth but no liquefaction; on potatoes the colonies are brown. This yeast produces alcoholic fermentation in beer wort and ferments maltose. It develops between 15 and 40° C. It is pathogenic for animals, but only for a time. Cultures sterilized by heat are toxic for guinea pigs.

[1] Maffuci and Sirleo. Osservazioni ed esperim. intorno ad un Blastomiceti, patogeno inclusione dello stesso nella cellula dei tessuti patologici, Policlinico. 1895.

CRYPTOCOCCUS PLIMMERI. Costantin [1]

This yeast was encountered by Plimmer [2] in a large number of cancers. The cells are round (4 to 40 μ) with a double membrane. The cells may be isolated or united to the number of from two to sixty.

On liquid media (2% glucose broth and 1% tartaric acid) it forms a deposit at the end of a few days. On gelatin added to the same bouillon the growth is feeble with no liquefaction. On agar added to the same liquid the colonies are small, isolated, slightly rounded, white in the beginning and yellow in old cultures. On potato the yeast develops a thick layer, at first white and after a time a brownish yellow. This yeast is pathogenic for guinea pigs only on intraperitoneal injection.

CRYPTOCOCCUS CORSELLII (Corselli and Frisch). Neveu-Lemaire

Isolated by Corselli and Frisch [3] from a sarcoma in the mesenteric ganglions of a man, this yeast possesses black cells of variable dimensions, slightly rounded, and agglutinated in masses. It is easily cultivated on gelatin, agar, dextrose, broths, sugar jellies, neutral or alkalin. It possesses a very feeble power of fermenting, and shows a pathogenicity for guinea pigs, dogs and rabbits in intraperitoneal injections.

CRYPTOCOCCUS DE GOTTI AND BRAZZOLA

Syn.: ATELOSACCHAROMYCES DE GOTTI AND BRAZZOLA. De Beurmann and Gougerot

Discovered by Gotti and Brazzola [4] in a myxosarcoma of the nasal passages of a horse, this yeast possesses cells of variable dimensions, round or slightly oval, with granular contents surrounded by a double membrane and a mucilaginous capsule, sometimes stratified.

In bouillon it produces clumps and on gelatin stabs a train of clumped masses with indented edges. On gelatin plates the colonies

[1] Costantin, Les levures des animaux, Bull. de la Soc. mycologique de France. v. XVII. 1901.

[2] Plimmer, Vorläufige Notiz über Gewisse von Krebse isolierle Organismen und deren pathogene Wirkung in Thieren, Centr. f. Bakt., v. XXV, 1899.

[3] Corselli, G., and Frisch, B. Blastomiceti pathogene nell'uomo. Annali d'Igiene sperim., v. V, 1895, and Centr. f. Bak. v. XVIII, 1895.

[4] Gotti and Brazzola, Sopra un caso di blastomicosi nasale in una cavalla. Memorie d. R. Ac. d. Scienze di Bologna, v. VI, 1897.

are white, becoming grayish yellow after a long time. Acid and glucose gelatin is liquefied. On glycerin agar the culture is creamy with indented borders. On potatoes the growth consists of a thick, creamy, white layer which becomes brownish in old cultures. This yeast is pathogenic for guinea pigs but not for other animals.

CRYPTOCOCCUS HOMINIS COSTANTINI (Costantin). Vuillemin

This yeast was isolated by Costantin [1] from a cancerous tumor of the breast. It possesses round cells and is distinguished from *Crypt. lithogenes* (San Felice) in that its cultures become brown when old and from *Sacch. tumefaciens* (Busse) because its membranes never become thick on ordinary media.

CRYPTOCOCCUS KLEINII. Erich Cohn

This species was discovered by Klein in milk, in which it was accompanied by various pathogenic bacteria. It has since been found by Erich Cohn.[2] The cells are globular, from 2 to 6 μ in size, with homogeneous contents, thin membrane, and surrounded by a hyalin capsule. The capsule persists but becomes smaller in cultures. This yeast is easily cultivated on beer wort agar. It does not ferment dextrose, maltose, or lactose nor liquefy gelatin.

CRYPTOCOCCUS ANOBII. Escherich

This species was found by Escherich [3] in the cells of the intestinal wall of the larva of *Anobium paniceum*. The cells are pear shaped or club shaped, from 3.5 to 4 μ in size, with center provided with refractive granules (Fig. 153). In culture it forms a pseudo-mycelium made up of cells shaped like a sausage.



Fig. 153. — *Cryptococcus Anobii* (after Escherich) *in situ.*

This yeast is easily cultivated in .01% of saccharose, liquid or solid (gelatin or agar). On gelatin it gives round colonies with no liquefaction.

[1] Costantin. Les levures des animaux. Bull. de Mycologie de France, v. XVII, 1901.

[2] Erich Cohn. Unters. über eine neue thierpathogene Hefeart (Hefe Klein). Centr. f. Bak., v. XXXI, 1902.

[3] Escherich. Ueber das regelmässige Vorkommen von Sprosspilzen in den Epithemis Käsers. Biol. Centr., v. XX, 1900.

CRYPTOCOCCUS PARASITARIS (Trabut) Vuillemin[1]

Syn.: SACCHAROMYCES PARASITARIS. Trabut

Discovered by Trabut on the grasshopper (*Acridium peregrinum*), upon which it is a parasite, this species has round cells, 3 to 4 μ, provided with refractive droplets. It does not ferment dextrose.

CRYPTOCOCCUS PSORIARIS. Rivolta

Syn.: SACCHAROMYCES PSORIASIS. Cattaneo

This yeast, encountered by Rivolta[2] in a case of dermititis, has round cells from 28 to 30 μ, a double membrane, and is often united in chains of 6 to 8 cells.

CRYPTOCOCCUS CAPILLITII. Vuillemin

Syn.: SACCHAROMYCES CAPILLITII. Oudemans and Pekelharing

This yeast has been described by Saccardo[3] as a spherical yeast from 2.5 to 8 μ in diameter, of a homogeneous color, with a thick membrane. It seems to bud. Blanchard considers it an *Oomycete*, and Guegen thinks that it is more closely related to the Algae.

CRYPTOCOCCUS OVALIS. Vuillemin

Syn.: SACCHAROMYCES OVALIS. Bizzozero

This organism was discovered by Malasses. The cells are shaped like a gourd (3.3 to 3.5 $\times$ 2.3 to 2.6 μ). They are made up of a large part surmounted by a bud. The membrane is thin and the contents include a large brilliant granule. Bizzozero considers this parasite as a yeast and gives the name of *Saccharomyces ovalis* to it. It has not been secured in culture. It was found in association with the preceding species and according to Saccardo may be another form of it. The recent researches of Dold[4] seem to indicate that this organism may not be a yeast but a bacterium.

CRYPTOCOCCUS CAVICOLA. Arthault

This species has been found by Stephen Arthault[5] in a pulmonary cavity. It is cultivated on potato and on agar, giving moist colonies,

[1] Guégen, F., Les champignons parasites de l'homme et des animaux, Thèse agrég. Pharmacie, Paris, 1902.

[2] Rivolta, Parasiti vegetali, 18–73.

[3] Saccardo, P. A., Sylloge fangorum, v. VII, p. 921.

[4] Dold, H. On the so-called Bacillus (Dermatophyton Malasserzi). Parasitolog. 3, 1910.

[5] Arthault, Flore et faune des cavernes pulmonaires, Arch. d. Paras., 1899.

thick, with a reddish color. They resemble very much the cultures of *Bacillus prodigiosus*. The cells are small and oval, from 8 to 12 μ long. This yeast is closely related to *Crypt. glutinis* and may perhaps be a variety of it.

CRYPTOCOCCUS NEOFORMANS. San Felice [1]

This yeast was found by San Felice on fermenting fruits. The cells are of variable dimensions; some of them have a refractive granule in the center. In the small cells the center is homogeneous. In the large ones one finds a central hyaline part with a very refractive peripheral ring.

This yeast develops on ordinary substances. On gelatin plates the surface colonies are different from those down in the media. The surface colonies are large like the head of a pin. They are quite round and form projections on the surface of the media. The deep colonies are somewhat spherical with a very fine contour. Gelatin is not liquefied. The colonies on agar have the same appearance. Growth on gelatin stabs develops as much along the line of inoculation as along the surface. There is no liquefaction.

This yeast is pathogenic and causes tumors in animals.

CRYPTOCOCCUS OF CLERC AND SARTORY [2]

This species found in chronic angina has elongated oval cells, from 7 to 10 μ by 5 μ in size, isolated or in groups of five or six. Budding may take place at one of the ends. The cells easily take various colors and are not decolorized by the Gram method of staining. The optimum temperature for budding is 30° C. The yeast vegetates easily on all the usual media and especially on slices of carrot. It does not liquefy gelatin, coagulates milk, and ferments lactose but not galactose. It secretes invertase, produces alcoholic fermentation of dextrose, but does not hydrolize starch. Possessed of a very feeble virulence, it is able under certain conditions, to live in the animal organism and cause localized and curable lesions.

BLASTOMYCES HESSLERI. Rettger

Found by Rettger [3] in an abscess on the chin, this yeast develops quickly in most of the media at blood heat, but contrary to ordi-

[1] San Felice, F., Ueber die pathogene Wirkung der Sprosspilze, Zugleich ein Beitrag zur Aetiologie der bösartigen Geschwülste. Centr. f. Bakt. v. XVII, 1895.

[2] Clerc, A., and Sartory, A., Étude biologique d'une levure isolée au cours d'une angine chronique, C. R. de la Soc. de Biologie, v. XIX, 1908.

[3] Rettger, A. Contribution to the study of pathogenic yeasts, Centr. f. Bak., v. XXXVI, 1904.

nary yeast, it does not prefer an acid medium. It resembles *Cr. Kleinii*, but differs by a certain number of characteristics which caused the author to regard it as a new species. This yeast is pathogenic to animals.

CRYPTOCOCCUS RUBER. Vuillemin

Syn.: SACCHAROMYCES RUBER. Demme

This yeast was isolated by Demme [1] from cow's milk, from the urine of a diabetic man and from diarrhoeic stools of an infant fed on milk. It forms a red layer on the sides of wooden pails which are used for milking. Demme has found it on dry leaves from Hayti. It was studied later by Casagrandi.

It is a yeast, round or slightly oval (Fig. 154), with a red or raspberry color. On gelatin it gives elevated growth. At the beginning the gelatin is not liquefied, but this change is accomplished in about eight months, according to Casagrandi. According to Demme, *Cr. ruber* provokes an alcoholic fermentation, but this property is soon lost by continued culturing on alkalin media. Casagrandi has not been able to observe it.

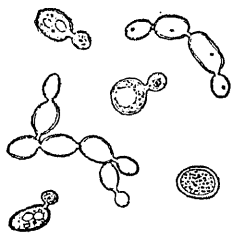


Fig. 154. — *Cryptococcus ruber* α, Chlamydospore (after Vuillemin).

This yeast develops easily on glucose or glycerol agar and on potato. The optimum temperature for budding is situated between 18 and 22°.

According to the researches of the above-mentioned investigators *Cr. ruber* is pathogenic. Introduced into the alimentary canal it produces symptoms of gastric enteritis. Subcutaneous or intraperitoneal injection causes the formation of a tubercle. Vuillemin [2] has shown that the fungus isolated by Bra from different cancers is related to *Cr. ruber*, and this latter seems to be related to *Cr. cavicola* of Stephen Arthault.

CRYPTOCOCCUS GUILLIERMONDI. Beauverie and Lesieur [3]

This was isolated by Guilliermond and Lesieur from human sputum during the course of a secondary cancer of the lungs. This yeast

[1] Demme, R., Saccharomyces ruber, Ann. de micrographie, 1889, and Annali d'Igyene sperim., v. XVII, 1897.

[2] Vuillemin P. Cancer et tumeurs végétales. Bull. des séances de la Soc. des Sciences de Nancy, 1900.

[3] Beauverie, J., and Lesieur, C. Etude de quelques levures rencontrees chez l'homme dans certains exsudates pathologiques. Jour. physiol. et pathol. generale. 14 (1912) 983–1008.

exists as solitary cells or grouped in two; they are round or oval with a thick membrane without a capsule (3–5 μ × 2.8–4.3 μ).

In old cultures, the cells often present abnormal forms, either sausage shaped or in chains, the cells of which are capable of branching into a rudimentary mycelium; there are also a number of giant cells present. The yeast develops easily and abundantly in most nutritive media. On carrot, small, round colonies are produced which become confluent but irregular and viscous. On potato, the growth is feeble with very small, white, dry colonies.

On agar plates, vegetation is abundant as a viscous layer with irregular edges becoming yellow. There is no liquefaction.

On fruit juices, glucose or saccharose solutions, there is feeble development as sediment. No scum is formed. On Raulin's fluid, there is scant development as a deposit with spherical cells (3.5 × 6.4 μ).

CRYPTOCOCCUS LESIEURI. Beauverie and Lesieur

This yeast was isolated from an ulcer of the stomach during a complication with typhoid fever. On beer wort, it has very small rounded or oval cells (2–3 μ). These may elongate and become curled. The elongated cells are also found united in filaments. On beer wort agar, the yeast shows a white creamy colony with the surface finely folded. It develops at 27–37° C. On beer wort after 9 hours there is a delicate ring with floating islands of scum. Only dextrose is fermented. Animal inoculation has not yielded positive results.

CRYPTOCOCCUS SULFUREUS. Beauverie and Lesieur

This yeast was isolated from a pharyngeal exudate during an attack of typhoid fever. On carrot, the cells are elongated, sometimes round (2–8 μ in diameter). This yeast develops well at 25° C.–37° C. There is a ring formed on beer wort after 23 hours. Dextrose, lactose and saccharose are fermented slightly. On wort agar, the colonies are white with a shiny surface and rounded edge.

CRYPTOCOCCUS ROGERI. Sartory and Demanche

This yeast was isolated from a peritonitis caused by a perforation of the stomach. The cells are long (3–10 × 2–3 μ).

This yeast is pathogenic for the rabbit and guinea pig. It vegetates on most of the culture media. It was also found by Beauverie and Lesieur in the pharyngeal exudate of typhoid fever.

CRYPTOCOCCUS SALMONEUS. Sartory

This species was found by Sartory [1] with *Oidium lactis*, in various specimens of gastric contents in hyperacidity. Of 17 of these juices 13 gave cultures of this yeast. It is a yeast quite closely related to *S. rosaceus* with a beautiful deep rose color in which the tint varies with the temperature and the culture medium. The cells are spherical, averaging from six to eight microns in diameter.

The optimum temperature for budding is situated between 22 and 25° C.; however, the yeast develops from 15 to 34°. In this latter case the rose color turns to a pale tint and becomes very feeble at 39°. Between 40 and 41° the yeast ceases to vegetate.

This yeast forms a rose-colored scum on glycerol broth at temperatures between 15 and 38°. The most favorable temperature for the formation of scum is between 26 and 28°. The cells of young scums differ a little from the cells in the sediment, but in old ones they become elongated or sausage shaped and one begins to notice structures like a mycelium. The deposit at the bottom of the culture flask is made up almost entirely of spherical cells only.

Cr. salmoneus is easily cultivated on all solid media (gelatin, agar, potato, carrot). It also develops easily on the various liquid media. The pigment is soluble in carbon bisulfide, benzine, chloroform, ethyl alcohol, ether, acetone and is insoluble in methyl alcohol.

This yeast secretes invertose but does not produce alcoholic fermentation. It is without action on dextrose, maltose, d-galactose, starch or inulin. It precipitates caseine in 18 days but does not peptonize the curd. It is not pathogenic for guinea pigs, rabbits and dogs.

LE DANTEC'S [2] YEAST

It was discovered in a stool from Sprue (chronic diarrhoea of a warm climate) and according to Le Dantec seems to be the cause of this sickness. The yeast ferments glucose broth and does not liquefy gelatin. In aerobic media it grows especially like yeasts; cultivated in anaerobic media it presents somewhat the form of a mycelium.

SACCHAROMYCES MEMBRANOGENES. Steinhaus [3]

This yeast was found by Steinhaus in a child attacked by scarlatina who presented the phenomena of tracheal stenosis. Fragments of

[1] Sartory, A., Cryptococcus salmoneus, Bull. de la Soc. myc. de France, v. XXIII, 1907.

[2] Le Dantec, A., Presence d'une levure dans la Sprue, C. R. Soc. Biol., v. LXIV, 1908.

[3] Steinhaus, F., Untersuch. über eine neue Menschen und Thierpathogene Hefeart, Centr. f. Bakt.. v. XLIII, 1906.

membrane taken from the trachea gave on inoculation the culture of this yeast.

It is a yeast with round cells, globular in shape, with a reddish pigment, and sometimes it is very large. Often the cells are pear shaped and bud. The bud appears at one end of the cell, which becomes pointed, but the budding is also accomplished as in other yeasts at some other point on the cell. The cells have a double wall, very fine and granular contents, with sometimes one or two bright granules. In membranes taken from the trachea, the cells are surrounded by a large capsule; this capsule appears in cultures freshly taken from the organs attacked and in very old cultures, but in general does not exist in cultures.

This yeast develops easily on acid substrate, especially on agar and beer wort. At the end of 12 days it forms white colonies, moist, round, confluent and becoming brown in old cultures. In peptone broth it causes a cloudiness and later a delicate precipitate in the bottom. Still later it causes a flocculent deposit at the bottom of the culture flask. On gelatin streaks it produces a white, moist growth; on stabs the growth is established along the line of inoculation with fine, round, isolated colonies. On the surface it forms a sort of a button. On gelatin plates the colonies are round and white. On dextrose agar there is a production of gas, but no gas is formed in manose, maltose or saccharose agar. On beer wort the growth consists of a dry grayish-white layer of round confluent colonies.

This yeast is very pathogenic for rabbits.

ATELOSACCHAROMYCES OF HUDELO. De Beurmann and Gougerot

This yeast was found by Hudelo, Duval and Loederich [1] in a human Saccharomycosis, manifested especially by a periostitis of the tibia. The cells are refractive, spherical (2–20 μ in diameter), sometimes oval or elongated into short sausage-shaped cells. No filaments are formed.

The species grows easily in ordinary media, especially on carbohydrate media with slight acid. The optimum temperature is situated at about 22°, but growth is accomplished even up to 38°. On carbohydrate agar white streaks are formed which are opaque and moist; on gelatin there is meager development with no liquefaction; on potato whitish streaks are formed, later becoming ochre colored, and finally a reddish black pellicle is formed.

This yeast inverts saccharose, but does not decompose lactose.

[1] Hudelo, Duval and Loederich, Un cas de Blastomycose a foyers multiples. Bull. et mem. de la Soc. de med. des hop. de Paris, 1906.

It does not ferment dextrose or maltose. It is pathogenic for mice, less pathogenic for rats, guinea pigs, rabbits and dogs. Intraperitoneal injection causes fatal septicemia in mice.

ATELOSACCHAROMYCES OF BREWER AND WOOD

De Beurmann and Gougerot

This yeast was isolated by Brewer and Wood from a human blastomycosis. *In situ* the cells are spherical (10–25 μ in diameter) and are surrounded by a large mucilaginous capsule. In culture no filaments are formed, but the cells sometimes remain united in short chains in old cultures. On glycerol agar the growth is small and gives grayish white colonies. On agar plates development is very abundant on the surface in a creamy yellow mass. On potato growth is difficult. On gelatin there is no liquefaction. The species grows difficultly in liquid media and produces no fermentation.

ATELOSACCHAROMYCES HARTERI (Harter[2])

De Beurmann and Gougerot

This yeast was isolated by Harter from a generalized human saccharomycosis. The cells are oval or elliptical, rather spherical (4–6 μ by 3–5 μ). On solid media and on Raulin's solution sometimes elongated units are observed, but never filaments, properly speaking.

On old cultures on carrot certain cells become round and very voluminous (5–8 μ in diameter). These are probably durable cells or chlamydospores.

The yeast grows well at 37° at laboratory temperatures. The growth ceases, however, at about 10°. The cells withstand 55°, but are killed in a quarter of an hour at 65° in moist condition.

On gelatin development is small and less abundant, white, granular, and penetrating into the medium with arborescent structure. There is no liquefaction on gelatin. On 1% glucose gelatin development is a little more abundant. On plain agar development is feeble and slow. On glycerol gelatin there is abundant growth with a production of a downy appearance at depths. On glucose or maltose agar there is abundant development of a white, creamy growth. On blood serum there is very meager growth. On carrot there is abundant development, quickly covering the whole surface with a creamy, white, thick, granular layer. On potato growth is grayish white, dry and not very abundant. The yeast inverts saccharose very slightly, but does not give any fermentation.

[1] Brewer and Wood, C., Blastomycosis of the spine. Annals of Surgery, 1908.

[2] Harter, G., De la blastomycose humaine. These de medecine. Nancy, 1909.

MERCIER'S [1] YEAST

This yeast was found by Mercier in the Blattes (*Periplaneta orientalis*) in which the cells existed in the adipose tissue under the form of round units, sometimes oval, with a very finely developed membrane. The parasite grows on bouillon and gelatin media. The colonies are white. The optimum temperature for budding is from 22 to 25° C.

SACCHAROMYCES CONOMELI LIMBATI. Karel Sulc [2]

This yeast has been found by Sulc in the pseudovitellius of an Homoptera *Conomelus limbatus*. The cells are elliptical or oval, some biscuit shaped having an alveolar content with small metachromatic granules in the vacuoles, and a little central or parietal nucleus. The buds form toward the end. The cells are often united two by two.

Sulc has also found *Sach. pseudococci farinosi* which lives in the pseudovitellius on another Homoptera, *Pseudococcus farinosus*. These yeasts probably live symbiotically with the insect.

A great many other yeasts have been described in different infections of men and animals; for instance, Maggiora and Gradenigo have isolated in a case of otitis *S. roseus;* Domingos Freire has observed in a case of yellow fever the presence of *Cr. xanthenicus;* Flava has found in a case of variola the *Cr. albus*. On the other hand Goetano has isolated the *Cr. septicus* which causes a rapid fatal septicemia in guinea pigs. Castellani has described in various tropical blastomyces *S. cantliei, Samboni,* and *Krusei,* also *Cr. Lowi*. San Felice has isolated *S. canis I* and *II* which provoke tumors in a dog. Finally, Dangeard has pointed out in the bodies of *Anguillules* the *S. anguillulae* which causes in these animals a very deadly malady. However, the morphological and biological characters of these yeasts have not been described; for that reason there will be no description of them at this time.

[1] Mercier, L., Un organisme a forme levure, parasite de la Blatte. C. R. de la Soc. de Biol., v. LX, 1906.

[2] Karel Sulc, Pseudovitellius und ähnliche Gewebe der Homopteren sind wohnstätten symbiötischer Saccharomyceten, Sitzungsberichte der König. Böhm. Gesellsch. der Wissenschaften in Prag, March 30, 1910.

CHAPTER XIII

FUNGI RELATED TO THE YEASTS

IT is deemed advisable to consider, at this time, a few of the fungi belonging to the family *Endomyces*[1] or in a doubtful position such as the *Monilia* and *Pseudomonilia;* these are set apart from the yeast by the greater complexity of their mycelium but the physiological and certain of their morphological characteristics resemble closely the *Saccharomycetes* from which, at times, they are separated with difficulty.

ENDOMYCES ALBICANS. Vuillemin

Syn.: APOROTRICHUM GRUBY OIDIUM ALBICANS. Robin. — SYRINGOSPORA ROBINI. Quinquaud. — SACCHAROMYCES ALBICANS. Reess. — MONILIA ALBICANS. Plaut. — DEMATIUM ALBICANS. Laurent

This fungus, which causes a sickness known as thrush, has been regarded in turn as an *Oidium,* a *Monilia* and a yeast. Since the work of Vuillemin it has been regarded as a member of the genus *Endomyces. In situ* and in cultures, *Endomyces albicans* has somewhat the same characteristics. It possesses a mycelium with cross walls and branches more or less well developed; yeast structures result by budding from the branches. (Fig. 155, 1 and 2.) The mycelium never acquires a marked differentiation and *Endomyces albicans,* speaking generally, is close to the yeasts. Both structures, yeast-like and mycelium, are able to change one into the other. The filaments seem able to form the yeast-like bodies and these latter the filaments. Mycelium formations are more or less well developed, depending on the conditions. In certain cultures the yeast-like structures predominate

[1] The genus *Endomyces* is characterized by a typical branched mycelium with cross walls, forming yeast-like bodies or oidia and chlamydospores, and with asci containing 4 ascospores. These are formed always at the expense of cells in the mycelium, most often at the end of a branch, exceptionally in some cell in the mycelium. In certain species, the formation of the asc is preceded by a copulation iso- or heterogamic. The genus *Endomyces* is differentiated from the Saccharomyces by the formation of a typical mycelium and the formation of asci in mycelial cells and never in the yeast-like structures. However, certain species have (*End. javanensis*) intermediate characters between the *Endomyces* and *Saccharomycetes* which makes it difficult to class them with either one of these two families.

with the mycelium reduced to its simplest form. In other media the filaments are most common. Cultures on slices of carrot have quite a development of mycelium while in Raulin's solution growth is almost solely of the yeast-like structures. According to Roux and Linossier,[1] the yeast structure is the normal one while the mycelial form appears only under conditions which reduce the vitality. According to Vuillemin, on the contrary, the filamentous form is the one which is normal and the yeast-like form appears only under bad conditions of food supply. The yeast-like structures are spherical, oval or elongated, and of variable dimensions. On Raulin's solution they become rather large and appear as large spherical cells somewhat resembling those of *S. cerevisiae* (Rajat [2]). Guilliermond has shown that the units of the filaments contain ordinarily a single nucleus, rarely more, and the yeasts are always uni-nuclear. This has been confirmed by H. Penau.[3]

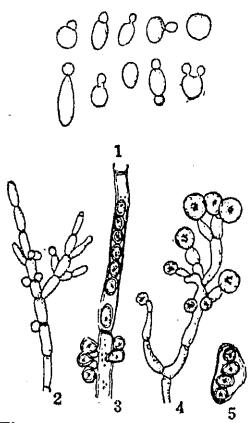


Fig. 155. — Yeast Forms from a Case of Thrush.

2, Mycelial Forms from a Case of Thrush; 3, Internal Globules; 4, Chronispores; 5, Asces (after Vuillemin).

Roux and Linossier, and later Vuillemin, have established in old cultures the production of very resistant forms comparable to chlamydospores. These, which have received the name of chronispores or chlamydospores, develop at the end of certain filaments in the form of distended cells filled with glycogen and surrounded with a thick membrane with three superimposed layers (Fig. 152, 4). Changed to different media, these chlamydospores germinate and produce yeasts or filaments.

Vuillemin has described, on the other hand, internal globules, (Fig. 155, 3) absolutely analogous in appearance to yeasts, which form on the interior of the filaments. The author considers them as resistant forms.

In our opinion, these internal bodies may be similar to those which are commonly found among the fungi. There are, here and there, the formation of yeasts or conidial forms, in the interior of an intercalary unit, with degenerating contents, by the budding of a contiguous unit. This latter buds in the interior of a dead unit which is near

[1] Roux, G., and Linossier, G. Rech. morph. sur le champ. du Muguet. Arch. de Med. experim. 1890.

[2] Rajat, H., Le champ. du Muguet. Thèse de doct. en medécine, Lyon, 1906.

[3] Penau, H., Cytologie de L'End. albicans forme levure, v. CLII, 1900, and Cytologie de l'End. albicans forme mycelienne. C. R. Ac. des Sciences, v. CLII, 1910.

it; the cells which are derived from the budding live parasitically on the protoplasm about it until their growth breaks it. Analogous formations are frequent among the Endomycetes. Rose has described them in *Endomyces magnusii*.

The ascs of *E. albicans* were accidentally discovered by Vuillemin on old cultures on beets, without which this author would have been unable to determine the conditions for their formation. The ascs appear as large, oval or elliptical cells, 4–5 μ in diameter, formed by lateral budding, or at the terminal of the units of the mycelia, or sometimes derived by germination of the chlamydospore. They possess membrane enclosing four flattened ascospores, slightly kidney shaped, with thick walls (Fig. 155, 5). The germination has not been observed. The presence of these ascospores has allowed Vuillemin to classify the fungus for thrush in the genus *Endomyces*.

These ascs have only been observed by Vuillemin and Daiereuva.[1] All the authors who have searched since to obtain them, have failed in their attempts. Also certain authors have thought that there might exist many varieties of *E. albicans*, some of which have preserved their sporogenic properties (Guegen, Rajat).

This opinion seems to be still further confirmed. Rajat has isolated three varieties of the fungus of thrush. One of these corresponds by its morphological and chemical characteristics to that species described by Vuillemin although it has not shown the formation of ascs. The other two types present morphological characteristics very different from the type species.

Beauverie and Lesieur have isolated from the blood of a fatal septicemia a variety of *Endomyces albicans*. This is distinguished from the type species by the fact that it ferments lactose and exhibits different cultural characteristics on carrot. Castelanni[2] has more recently shown the plurality of the thrush fungus. He has isolated 29 different fungi from thrush cases. He also separated a number of new races of the thrush fungus.

All of these fungi belong to the genus *Monilia* and may be differentiated by their biochemical characteristics. Guilliermond isolated three types of the thrush fungus from infections at hospital No. 101 during the war, at Lyon. Two of these belonged to the genus *Monilia* and the third was a typical saccharomyces, with ascs but not corresponding to *Saccharomyces anginae* of Troisier and Alchalme.

[1] Daiereuva, M., Rech. sur le champ. du Muguet. Thèse de médecine Nancy. 1899.

[2] Castelanni, A. The plurality of species of the so-called thrush fungus (Champignon du muguet) of temperate climates. Annals de l'Institute Pasteur 30 (1916), 149.

E. albicans develops between 20 and 39° C. and grows on solid or liquid media slightly acid; no scum is produced on the surface of liquid media. On carbohydrate liquid media and fruit juices it gives a slight growth with a flocculent sediment. On gelatin plates the colonies are round, white and creamy, and it produces liquefaction of the gelatin. In gelatin stabs development is slight and superficial. On agar the fungus produces a white line which thickens to a creamy layer at first thick then honeycombed. On potato it gives small colonies of a dirty white color and on carrot creamy white and folded growth. It grows with difficulty in milk, which it coagulates in 20 to 30 days.

E. albicans causes a slight fermentation of dextrose.

Anderson has mentioned the very frequent presence in the human intestines of a fungus very closely related to *Endomyces albicans* to which he has given the name of *Parasaccharomyces Ashfordii.*

PARASACCHAROMYCES ASHFORDII. Anderson [1]

"*Morphology.* In young cultures cells are round or slightly oval; in old cultures cells are of many forms: oval, elongated, elliptical, round, or irregular; giant cells are common. Septate mycelium develops in gelatin hanging-drop and in old cultures. Budding occurs from any point on the young cells, but usually near the ends of articles in old cultures. The size is $4.5 \times 5\,\mu$.

" *Cultural Characters.* On glucose agar the streak is filiform, raised, glistening, chalk-white and smooth; later the central portion may become rugose or pitted; the edge of the streak may remain entire or may become decidedly filamentous, due to the outward growing hyphal elements under the surface of the medium. There is a growth in gelatin stab at first filiform, later it develops scattered, bushy clusters of filaments. In liquid sugar mediums and beer wort a very evident ring formation occurs; no pellicle is present.

"*Physiologic Properties.* It ferments glucose, maltose and levulose; occasionally sucrose and galactose are fermented. Yeast-water sugar mediums, with an initial acidity of + 1, become more alkaline. Litmus milk is rendered alkaline in 2 weeks, but is not clotted. Gelatin is rarely liquefied.

"The culture was isolated from a sprue patient by Dr. B. K. Ashford in Porto Rico.

" This species strongly resembles the fungus variously called Oidium albicans, Monilia albicans, and Endomyces albicans. Castellani ('16) has, however, reserved the name Monilia albicans for a species which

[1] Anderson, H. W. Yeast-like fungi of the human intestinal tract. Jour. Infectious Diseases 21 (1917) 341–386.

always clots milk and liquefies gelatin. *Monilia albicans*, *Oidium albicans* and *Endomyces albicans* are synonyms, and if Vuillimin's ('99) results are accepted and are of general application to all of these, the correct name for the species is Endomyces albicans, since he states that this species forms asci after the manner of other species of the genus Endomyces. Since all efforts to develop the perfect stage of the sprue organism, both by Dr. Ashford and myself, ended in failure and since it differs in many of its physiologic characters from the typical Endomyces albicans, it has been thought best to give it specific rank rather than to regard it as a variety of Endomyces albicans."

PARASACCHAROMYCES THOMASII. Anderson [1]

"*Morphology.* In young cultures, cells are elliptical or ovate; in old cultures, surface cells are round, oval, elliptical, or elongated; submedial cells form a distinct mycelium mostly by elongation of cells produced by budding. There is occasional septation in gelatin hanging-drop. Budding occurs from ends or shoulders. The size is $3.5 \times 5\ \mu$.

"*Cultural Characters.* On glucose agar the streak is, at first, white, glistening, convex, and smooth; later the surface becomes rugose with a decidedly elevated ridge down the center. Beneath the surface of the medium the radiating hyphae form a villous fringe. In beer wort and liquid sugar mediums no pellicle or ring is present. In gelatin-stab cultures the growth is finely villous. Giant colonies in beer wort gelatin are decidedly yellow in color and otherwise very characteristic.

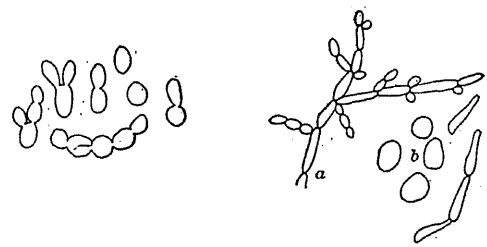


Fig. 155-B. — *Parasaccharomyces Thomasii*, Anderson.

[1], Cells from Young Beer Wort Culture; *a*, Elongated Cells Forming a Pseudo-mycelium Beneath the Surface of an Agar Slant; *b*, Cells from the Surface of the Same Culture.

"*Physiologic Properties.* Slow fermentation of glucose, levulose and maltose. In litmus milk there is a decided alkaline reaction.

"The culture was isolated from human feces.

"The species is similar to Parasaccharomyces Ashfordii in its physiologic properties. It differs mainly in its morphologic characters and the type of giant colonies produced. The yellow, rugose colony in beer wort gelatin is especially characteristic and easily distinguishes in this species from P. Ashfordii."

[1] Anderson, H. W. Yeast-like fungi of the human intestinal tract. Jour. Infectious Diseases 21 (1917) 341-386.

ENDOMYCES LINDNERI. Saito

This yeast was isolated from Chinese yeast by Saito. This Chinese yeast was used in the preparation of beer. It has the same morphological characteristics as *Endomyces fibuliger;* the ascs are often formed, as in *Endomyces liger*, from an anastomosis taking place between two cells in the mycelium. Maugenot has shown that these anastomoses are analogous to those which have been described for *Endomyces fibuliger* and never result from a copulation. They represent what is left of an ancestral sexuality. *Endomyces Lindneri* is very closely related to *Endomyces fibuliger* and is distinguished from this genus only by the fact that it ferments maltose and dextrose on which *Endomyces fibuliger* has no action.

ENDOMYCES HORDEI. Saito

Saito has isolated an organism which possesses certain characteristics of a *Monilia* with fragments of mycelium with cross walls forming yeast-like structures as conidia. When inoculated into media, these germinate into a budding mycelium. In the sediment, they develop especially as yeasts. The mycelium is also able to break up into fragments like oidia in old cultures. The ascs appear in old cultures on agar and gelatin. On plate cultures, they form in great numbers at the end of three days. They are formed by budding of the units of the mycelium under the form of large round cells (6–12 μ). The ascospores are to the number of from 2 to 4 per asc, and are hat shaped (3–4 μ). They are provided with an exosporium and an endosporium. During germination the exosporium breaks and the ascospore germinates by ordinary budding.

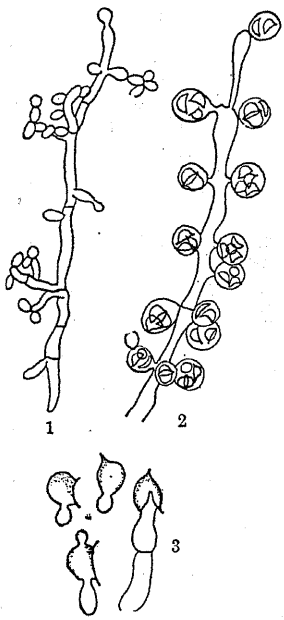


Fig. 155–C. — *Endomyces Hordei.*

1, Budding Mycelium; 2, Ascs; 3, Germination of Ascospores (after Saito).

The growth on plates is under the form of small moist patches with filaments. On beer wort or decoction of "Koji" the fungus forms a very thick scum which is moist and at the same time a small sedimental growth.

The optimum temperature for growth is 30° C. The yeast will

vegetate, however, between 15° and 40° C. It induces a fermentation in beer wort very slowly and gives off an ethereal odor. It acts energetically on saccharose, maltose, dextrose, levulose, mannose, galactose, raffinose, dextrine, xylose and arabinose and feebly on rhamnose and α-methylglucoside.

Endomyces Hordei resembles *Endomyces fibuliger very closely.* It is also related to *Endomyces Lindneri.* The formation of the asc by simple budding, without any trace of sexuality, is responsible for this resemblance.

ENDOMYCES CAPSULARIS. Guilliermond

Syn.: SACCHAROMYCES CAPSULARIS. Schiönning [1]

This yeast was described in 1903 by Klöcker who isolated it from pastureland in the Swiss Alps. Guilliermond finally subjected it to careful study.

Endomyces capsularis for the most part has cells in the mycelial and the yeast form at the same time. It vegetates at the bottom of the medium as a sediment or in the form of a scum. The mycelium is especially well developed in the scums or on solid media. It is branched, with cross walls, and according to the investigations of Guilliermond, always has a single nucleus. It is able to present different appearances. Some of the filaments remain sterile while others form by lateral and terminal budding numerous yeast-like cells. Rarely there are others which form small septa, dividing the thread into units which break off like oidia.

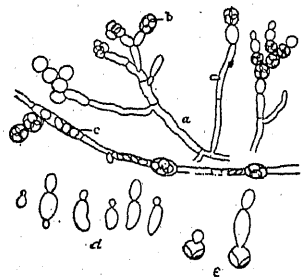


Fig. 156. — *Endomyces capsularis.*

a, Filaments with Ascs; *b*, With Branching at Ends; *c*, Filaments with Ascs in Intercalary Cells; *d*, Yeasts; *e*, Germination of Ascospores (after Schiönning).

The yeast-like bodies develop especially in growths of sediment. They look like true *Saccharomyces;* their form is ellipsoidal or oval like *Saccharomyces Pastorianus* or *Saccharomyces ellipsoideus* (Fig. 156 *d*). Many among them have a point at one or both ends. They never have but a single nucleus. Aside from the yeast-like structures, one may find some elongated or walled cells which represent yeasts in the process of making up a mycelium.

The optimum temperature for vegetation is situated between 25 and 28° C. The maximum temperature is 38.5° C. and the minimum about 0.5° C. The ascs appear under the same conditions which

[1] Schiönning, H. Nouveau genre de la famille des Sacch. Comp. Rend. du lab. de Carlsberg. Vol. 6, Book II, 1904.

determine the sporulation of yeast (plaster blocks, cultures in yeast water and slices of carrot). The optimum temperature for sporulation on plaster blocks is between 25° C. and 28° C., the maximum is 34.5°–35° C. and the minimum 5–8° C. The ascs always form at the expense of units in the mycelium and never of the yeast-like structures. Finally, they only appear in contact with air on solid substrates and in scums. The ends of the threads separate into cells which become round and produce spherical or elongated forms similar to oidia. These cells develop either by constriction or transverse partition or by a process intermediate between these two processes. The cells thus formed swell up and show a granular contents very refractive, later changing gradually into ascs (Fig. 58, 2 and 3, and Fig. 157, a). Often the ascs are able to form from an intercalary cell in the mycelium which enlarges and becomes round (Fig. 157, b). The investigations of Guilliermond [1] indicate that the ascs possess a single nucleus like those of the *Saccharomycetes*. A karyogamy does not take place here as with Exoascus. The ascs almost constantly possess 4 ascospores.

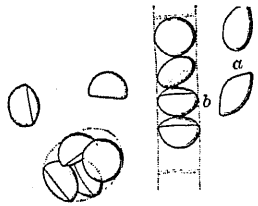
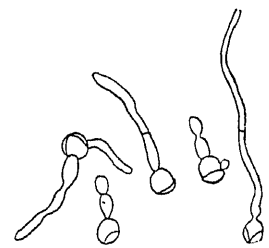


Fig. 157. — Ascospores in *Endomyces capsularis* (according to Schiönning).

The ascospores are very resistant to acids. If the mycelium which has produced ascs is treated with a strong solution of sulfuric acid or other mineral acids, the mycelium and the ascs dissolve. On the other hand, the ascospores resist and take on a beautiful red color. The ascospores of the Saccharomycetes are, on the other hand, strongly attacked by these acids and not colored a rose color. The ascospores are ellipsoidal or oval (3.5 to 8 μ in diameter). They possess a double membrane, an exosporium and an endosporium. The exosporium is formed of two valves in which the adjacent edges cause a sort of projecting ring by means of which the ascospores resemble those of *Willia Saturnus*. This ring separates the ascospore into two unequal parts (Fig. 158).


Fig. 158. — Germination of Ascospores in *Endomyces capsularis*.

Reaching the adult stage, the ascospores absorb their wall quite rapidly, setting free the ascospores, but these more often remain united in groups of four. When the ascospore germinates, the exosporium cracks to form two unequal parts which remain united at the point

[1] Guilliermond, A. Recherches cytologiques et taxonomiques sur les Endomycetees. Rev. gen. Bot. 21, 1909.

for some time. Germination of the ascospores is accomplished either by the formation of a germinating tube or by budding. The germinating tube becomes the point of beginning of the mycelium. (Fig. 156 e, and 158.) On beer wort the ascospores germinate by budding, only the yeast-like cells, which elongate without separating, show a tendency to form a mycelium, but never a true mycelium. In yeast water and on slices of carrot, on the contrary, ascospores are never formed from budding but a filament forms walls, thus forming directly a mycelium.

In certain unfavorable conditions for growth of this fungus, the ascospores may form a bud or a germinating tube which changes directly into an asc.[1]

Endomyces capsularis develops for the most part in artificial media. On beer wort at 25° C., after about one day, it forms on the surface a deposit of yeast which sets up the alcoholic fermentation. After two days, there are formed on the surface of the wort small floating patches of scum made up of a typical mycelium. After a prolonged repose the scum finally covers the whole surface; this scum is frequently situated above great bubbles of froth which make it uneven. If the culture is left in quiet repose, the scum forms a thick cover on the surface, very uneven, dry and white and slightly velvety, composed of a mixture of yeast-like structures and mycelium.

In yeast water, this fungus forms on the surface of the medium at the end of two days white islands of scum composed of a mycelium in which some of the mycelial threads form yeast bodies by constriction. After four days, the entire surface is covered with quite a thick mycelium, slightly velvety in appearance. Vegetation is then formed of a typical mycelium well developed, in which the ends produce many ascs. On must gelatin, a dry velvety growth is produced

[1] According to Klöcker and Dombrowski, the *Saccharomycetes* are distinguished from the *Endomycetes* by the fact that in the first the ascospores are able, under certain conditions, to change directly into a new asc without preliminary multiplication while in the second, the ascs only form after the formation of a mycelium. These authors think that this peculiarity makes a better differential characteristic between the *Endomycetes* and the *Saccharomycetes*. According to Klöcker, *Endomyces capsularis* may be classed among the *Saccharomycetes* because in this fungus the ascospores are capable of producing ascs in their germination. On the contrary, *Endomyces fibuliger* may be classed among the *Endomycetes* because the ascospores never change into ascs. Klöcker and Dombrowski have only observed these two endomyces and have not investigated whether the spores of other members might not change into ascs. Indeed, one ought not to attribute too much importance to the opinions of these two investigators, who base their statements on too hasty generalizations. What connects *Endomyces capsularis* to the *Endomycetes* is the high differentiation of its mycelium and the mode of formation of its ascs always at the expense of the mycelium. This fungus is, however, so closely related to *Endomyces fibuliger* that a separation is merely arbitrary.

with a grayish white color. The gelatin is not liquefied. On must agar, the growth is dry, much folded and velvety, assuming a chocolate color after a time. This fungus ferments maltose, dextrose, levulose, and d-galactose, but does not act on l-arabinose, raffinose, lactose and saccharose. It does not secrete invertase.

Schiönning has described this fungus as a Saccharomycete which he classes along with *Saccharomycetes guttulatus* in the genus *Saccharomycopsis* characterized with its ascospores in a double membrane. On account of the high differentiation of its mycelium and the formation of ascospores almost always at the end of the filaments of the mycelium and never at the expense of yeast-like cells, we have been led on the contrary to class it with *Endomyces fibuliger* and put it into the genus *Endomyces* under the name of *End. capsularis*. It will be demonstrated in the following paragraph that this fungus is very closely related to *Endomyces fibuliger*.

ENDOMYCES FIBULIGER. Lindner [1]

Endomyces fibuliger was discovered in 1908 by Lindner on bread where it formed white spots resembling chalk and caused a trouble known as "chalky bread." By the investigations of Lindner, Dombrowski [2] and Guilliermond,[3] this yeast is well known to-day. In cultures it has a typical mycelium with cross walls and branches in each unit of which there is a nucleus. The filaments of this mycelium at times form conidia, yeast-like structures and ascs.



Fig. 159.—Formation of Conidia in *Endomyces fibuliger*.

The conidia appear only in that part of the mycelium that is directly exposed to the air, that is, in the scum on the surface liquid media or in the upper reaches of the growth on solid media. They are formed in great abundance under these conditions and show a white powdery appearance. These conidia either form directly from the mycelium by budding, or form at the expense of budding cells like the yeasts which are formed by branches in the mycelium. (Fig. 159.) They separate from the units which form them and leave a sort of sterigmata which remains attached to the latter cells. The conidia look like grape seeds which are provided with a thick membrane, a protoplasm filled with fat

[1] Lindner, P. Endomyces fibuliger n. sp. Wochensch. Brau. No. 24. 1908.

[2] Dombrowski, W. Sur l'End. fibuliger. Comp. Rend. lab. de Carlsberg, Vol. 7, Book 4, 1909.

[3] Guilliermond, A. Recherches cytologiques et taxonomiques sur les Endomycetees. Rev. gen. Bot. 21, 1909; Remarques sur le develop. l'End. fibuliger. Comp. Rend. Soc. Biol. 67, 1910.

globules and a single nucleus. The outer part of the membrane is easily detached when the conidia separate from the cells which form them. The conidia have a tendency to reunite in small masses surrounded by bubbles of air. A sort of network, mucilaginous in character, is formed which is quite comparable to that formed by the Saccharomycetes. This network is destroyed by heat. Possibly it constitutes a means of preservation for the conidia.

The conidia never bud in the media in which they are formed. Only when transferred into a fresh medium is it that they bud either by yeast-like structures or by sending out a germinating tube which branches to form a mycelium. They represent, then, forms which are comparable to the Chlamydospores of other *Endomycetes*.

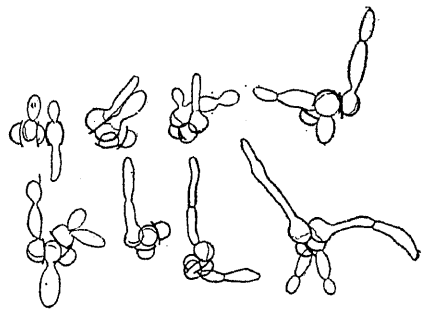


Fig. 160. — Germination of Ascospores in *Endomyces fibuliger*.

In parts of the mycelium which are situated in scantily aerated locations, as in the sediment in a liquid culture or deep down into a solid medium, the mycelium never produces conidia but, on the contrary, forms a large number of yeast-like structures. (Fig. 55.) These vary in their shapes and sizes. In certain media, they are smaller than conidia and resemble the *Mycoderma*. Sometimes, they may be much larger than the conidia and possess a round shape. They contain but a single nucleus. These yeasts, after being detached from the mycelium for a time, continue to bud in the medium in which they are formed and furnish new generations of the yeasts. On fresh media, they elongate and furnish a mycelium or germinate into yeast-like structures.

Often there may be seen in parts of the mycelium that form these yeast-like bodies, a sort of dissociation of filaments. The walls come nearer and the units which are thus formed separate into elongated cells which look like oidia.

Lindner has noticed, in certain cases, the formation in the intercalary units of the filaments, of internal cells which he ignores, but which seem to us to be conidia or yeast structures.

The asci are formed under the same conditions as with the *Saccharomyces*. They appear quickly if a piece of the mycelium, young and well nourished, is placed in a covered dish containing a thin layer of distilled water or on different solid media (slices of carrot) as well as in most old cultures. The most favorable temperature for sporulation

is situated at about 20° C. At this temperature the ascs appear in about 72 hours. Like the conidia, the ascs are always formed in the presence of air. They are able to appear in the same time and numerous filaments may be found which form both ascs and conidia. They form, as with *Endomyces capsularis*, at the ends of the filaments either by budding, or by partition, followed by a separation of terminal units which gives a chain of ascs. Sometimes they are formed by an intercalary unit.

The formation of these ascs is of special interest because of the anastomosis which takes place. In most cases the ascs are formed without anastomosis, as in the two preceding species of *Endomyces*, but in about half of the cases, the young bud destined to form an asc anastomoses with a cell situated in the vicinity by means of a sort of copulation canal. This phenomenon has been sufficiently described in a preceding chapter. Let us recall, however, that the middle wall that separates the asc from the cell with which it is anastomosing does not disappear but persists in the copulation canal. Sometimes this wall does disappear but even in this case there is produced no mixture of the contents of the two cells. (Figs. 56 and 57.) Also we should look on these anastomoses as traces of an ancestral reproduction analogous to that of *Eremascus fertilis*, but having disappeared today.

The ascs appear like large round or oval cells. They have but one nucleus and do not possess karyogamy. They contain numerous ascospores which may vary from one to four, but usually four. The ascospores are hemispherical and they are surrounded by a projecting ring which makes them look like a hat. In this manner they are like the ascospores of *Willia anomala* and *Endomyces decipiens*.

Germination of the ascospores has been recently studied by Dombrowski. The ascospores are provided with a double membrane, an exosporium and an endosporium. At the moment of germination, the ascospores take their usual form if in a young culture or become globular if in an old culture; the exosporium opens up at any place on the surface of the ascospore. These germinate indifferently either to produce yeast-like bodies or to form a mycelium directly. (Fig. 160).

The ascospores are incapable, as with *Endomyces capsulatus*, of yielding ascs directly. The ascs are only produced when the mycelium is well developed. *Endomyces fibuliger* develops quickly on most media. In beer wort, after three weeks, it shows a dry scum formed of mycelium, covered by numerous conidia which give it a farinaceous appearance; in the sediment, yeast-like cells are formed. In course of development, an agreeable aroma is given off with a feeble fermentation.

In wort gelatin, it develops with a dry farinaceous spot and brings

about a liquefaction of the gelatin after a week. The vegetation is made up of a thick mycelium which gives numerous conidia. In wort agar, the colonies are of a chocolate color. On carrot, this fungus develops abundantly with a mycelium which in the beginning furnishes many conidia; later after 18 days, there is an abundant production of ascs. The deep portions of the mycelium form many yeasts.

Endomyces fibuliger ferments saccharose actively and less actively dextrose, d-mannose and levulose; it ferments feebly raffinose, lactose, d-galactose and α-methylglucosides. It has no action on maltose, dextrine, arabinose, xylose, trehalose, melibiose, mannite and inuline. *Endomyces fibuliger* is, in general, closely related to *Endomyces capsularis*. It resembles it by the complexity of its mycelium, its yeast structures and the mode of formation of ascs and is distinguished only by the formation of conidia and the traces of an ancestral copulation which it has kept.

ENDOMYCES JAVANENSIS. Klöcker

This species was discovered in 1909 by Klöcker [1] in soil from Java. The vegetation is composed in part of cells like yeasts and in part of a mycelium with walls. The mycelium offers a slight tendency to separate its units like oidia. However, it is much more reduced than in *Endomyces capsularis* and *Endomyces fibuliger* (Fig. 161). The yeast cells (7 to 9 μ in length) often look like lemons but some look like a spindle; others are ellipsoidal, spherical, in the form of a sausage or very much elongated. In a general manner, they resemble the cells of *Saccharomyces apiculatus* very much. The temperature limits for growth are: maximum 36°–38° C., minimum, 5° to 10° C.

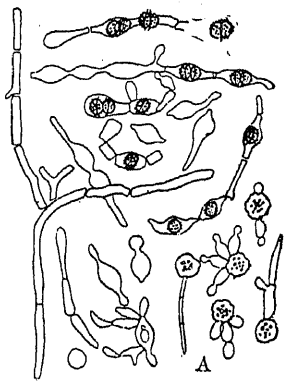


Fig. 161. — *Endomyces Javanensis*. Mycelium with Oidia, Yeasts and Ascs. — A. Ascospores in Process of Germination (after Klöcker).

Sporulation is abundant in liquid and solid media as well as on plaster blocks. The temperature limits for sporulation on plaster blocks are: maximum, 34–36° C., minimum, 5° to 10° C.

The ascs seem to form indifferently in the yeast cells or at the expense of some cell in the mycelium. They usually enclose a single ascospore, rarely two. The ascospores are ellipsoidal and have the

[1] Klöcker, A. L'Endomyces Javanensis, n. sp. Comp. Rend. Trav. du Lab. de Carlsberg. 8, Book 4, 1909.

shape of a slightly flattened ball. They have but a single membrane. This membrane is supplied with more or less distinct elevations. A projecting ring runs around the middle and makes the ascospores look like those of *Willia Saturnus*. However, this ring may be so placed that the ascospores look like hats. Germination of the ascospores is accomplished by budding in the yeasts and by the formation of germination tubes (Fig. 161, A). In old cultures in must, there is formed on the surface of the medium and along the walls a thick ring which may grow and entirely cover the surface. The least jarring of the flask causes this to fall to the bottom. The giant colonies on gelatin have a viscous appearance. The surface is much folded and the center slightly sunken by a slight liquefaction of the gelatin. This species does not invert saccharose or ferment dextrose; it acts feebly on levulose.

Endomyces javanensis constitutes a more immediate link between the *Saccharomycetes* and the *Endomycetes* than some of the other *Endomyces*. The fact that the ascs form often from yeast cells unites it very much more to the *Saccharomycetes* and accordingly it is advisable to class it with this family.

ENDOMYCES CRUZI. Mello and Paes [1]

Mello and Paes found a yeast with oval cells (4–8 by 2–4 μ) in the lungs of a man of 45 years who had been asthmatic for 10 years. This yeast grew well on glycerol and plain potato, Raulin's solution, Sabouraud's gelatin, plain and glycerol bouillon and alkalin agar. On solid media the growth was a yellowish creamy white. The growth was more abundant on alkalin media. This yeast fermented glucose, maltose, dextrose and saccharose. In cultures these authors observed both budding forms and mycelial cells. The ascs contained from two to four spores. This fungus seemed to be closely related to *Endomyces vuillemini*, Landrieu, 1912. The latter fungus, however, prefers an acid medium and does not ferment dextrose.

[1] Mello, F. de and Paes, A. Endomyces cruzi n. sp. agent (?) d'une endomycose bronchique simulant l'asthme. Arquivos de Higiene e Patologia exóticas, 6 (1918) 51–60. Bull. Past. Inst. 17 (1919) 636.

MONILIA CANDIDA. Bonorden [1]

Syn.: MONILIA BONORDENII. Vuillemin [2]

This species was described by Hansen,[3] and was isolated from fresh dung and fruit juices in which it forms a white layer. When put into wort, there is abundant growth of cells having the appearance of yeasts and resembling *S. ellipsoideus* and *cerevisiae*. (Fig. 162.) A strong alcoholic fermentation is set up during which the surface of the liquid is covered by a thick scum; this is made up of ordinary cells which elongate to make a mycelium. (Fig. 163.) According to the investigations of Hansen, this fungus forms 1.1 per cent of alcohol by volume during the time that *Saccharomyces cerevisiae* forms 6 per cent. But while *Saccharomyces cerevisiae* stops at this per cent, *Monilia candida* continues its action. After 6 months fermentation there is 5 per cent of alcohol by volume.

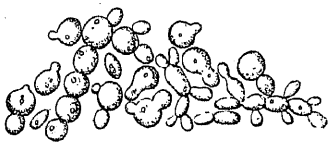


Fig. 162. — *Monilia candida* Young Cells in a Scum.

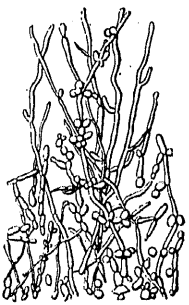


Fig. 163. — *Monilia candida.* Filamentous Forms in a Scum (after Hansen).

This yeast secretes invertase but it remains in the interior of the protoplasm and never diffuses through the membrane. Fischer and Lindner have found that it is impossible to extract this enzyme. They have, however, inverted saccharose with the dried fungus, even in the presence of antiseptic substances. Cells broken up with glass were also used. *Monilia candida* inverts maltose and ferments the dextrine (Bau). It easily withstands high temperatures. On account of this it may develop in solutions of saccharose and beer wort at 40° C.

Anderson has recently mentioned the frequent presence of a yeast resembling *Monilia candida* in the human intestinal tract. Lindner and Knuth have also found *Monilia candida* in epizoötic lymphangitis.

[1] The genus Monilia, created by Persoon, is quite badly characterized. It includes filamentous fungi characterized by the formation of oval conidia, elliptical or in chains (conidial yeasts or oidial forms).

[2] Vuillemin, P. Difference fondam. entre le genre Monilia et les genres Scopulariopsis, Acmosporium et Catenularia. Bull. Soc. Mycol. de France, 27, 1911.

[3] Hansen, E. C. Recherches sur la physiologie et morphologie des alcooliques ferments. VII, Action des ferments alcooliques sur les diverses espèces de sucre. Levures alcooliques à cellules resemblant à des Saccharomycetes. C. R. Trav. du lab. de Carlsberg, 2, Book 5, 1888.

MONILIA NIGRA. Browne

This Torula was isolated by Browne from raw sugar. One sample of such sugar, which had been sealed for three years, gave 1500 colonies

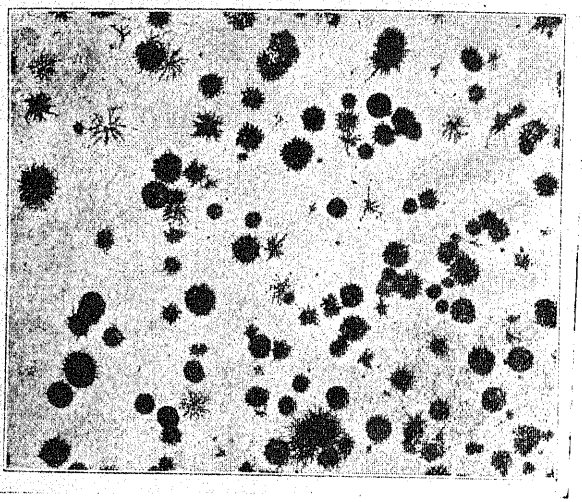


Fig. 163–A. — Small Colonies of *Monilia nigra* in Various Stages of Growth (after Browne).

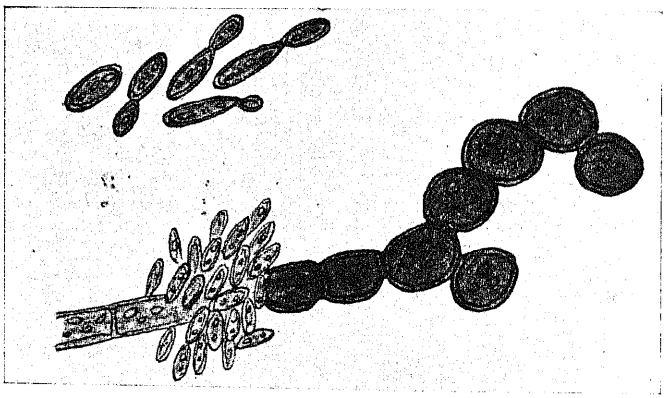


Fig. 163–B. — Magnified Cells of *Monilia Nigra.*
Below is the End of One of the Hyphae, Covered with Bud-Cells, and Terminating in a Cluster of Dark Conidia.

in 1 gram. This is one of the most destructive organisms found by this author in Cuban raw sugar. Browne describes the colonies on raw sugar agar as being, at first, small star-shaped dots which, under

[1] See reference for *Torula communis.*

the microscope, consists of radial hyphae. "The latter throw off a conglomerate of bud cells, the mass of which increasing in thickness soon gives the colony a starfish appearance. This primary growth is usually succeeded by a secondary growth, due to the propagation

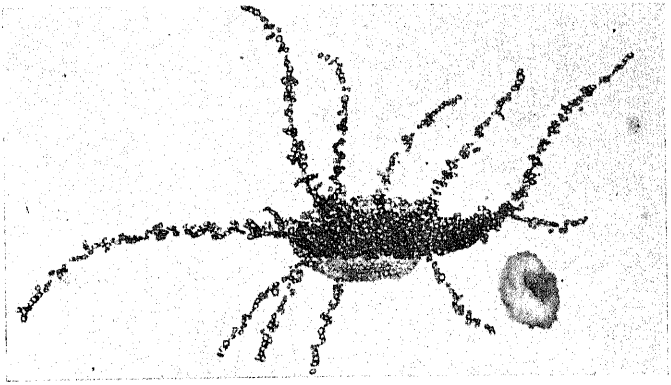


Fig. 163–C. — Magnified Colony of *Monilia fusca.*
The Radiating Hyphae are covered with Bud-Cells and Dark Conidia (after Browne).

of the bud cells, which, without the formation of hyphae, germinate like yeast and cover the center of the colony with a white amoeba-like film." When the colonies have attained a diameter of from 1 to 15 mm., the hyphae break up into clusters of dark conidia which give

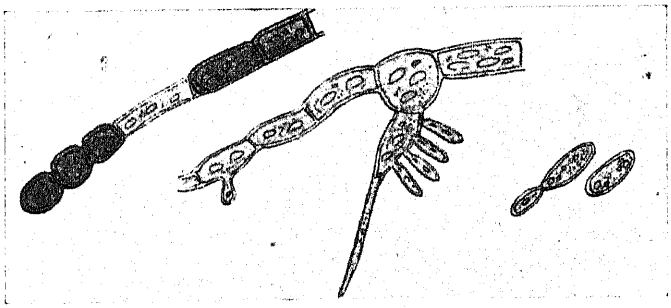


Fig. 163–D. — Magnified Cells of *Monilia fusca.*
In the middle is a branched part of the mycelium bearing 4 bud-cells; two of the latter (one germinating) are shown at the left. At the right is the end of one of the hyphae, breaking up at the end into 3 conidia and in the middle into 2 oidia (after Browne).

the colony a black color. This gives it the name of *Monilia nigra.* If the colony stops growing before the conidial stage is reached, no black color is assumed but the white remains. Under the microscope, the hyphae are of the ordinary branched type but more often are studded with clusters of bud-cells. These latter are elliptical in shape and may produce new hyphae, or propagate like a yeast. When the

hyphae are mature, they break up at the ends into thick-walled conidia. The disintegration of the hyphae into thick-walled cells may also occur at other places than the end. This gives them the appearance of oidia. The various cell units of this microörganism contain many oil globules. This *Monilia* grows well in raw sugar solutions except those which are most concentrated. The culture fluid becomes turbid with mycelium which, after several days, may extend up the sides of the tube. There is slight gas formation with a fruity odor. The action on the raw sugar consists principally in the inversion of sucrose. This inverting ability is restrained by raising the concentration of the raw sugar. No further description of this organism is given by the author.

MONILIA FUSCA.[1] Browne

The colonies of this *Monilia* are described by Browne as being similar to those of *Monilia nigra* except that the hyphae are much longer, show a less pronounced tendency to form the yeast-like structures, and have a greenish brown color, instead of black, in the conidial stage. The Monilia grows in raw sugar solutions except the most concentrated. The media become turbid with a deposit of mycelium and cells. The walls of the container to a distance of 2 cm. may be covered with a dark conidial growth. There is a slight formation of gas and a fruity odor. *Monilia fusca* possesses a stronger inverting action than *Monilia nigra*. Browne regards these *Monilia* as the most destructive organisms found in raw sugar on account of their ability to adapt themselves to different conditions in their environment.

GEIGER'S PSEUDOMONILIA[2]

Under the name of *Pseudomonilia*, Geiger has included a number of yeasts which will be described at this time.

Pseudomonilia albomarginata

The cells of this yeast are oval (4 to 6 μ) and have a protoplasm containing one or three refractive granules and a vacuole containing crystals. The mycelium is made up of long filaments. This yeast forms a folded scum. The giant colonies possess special forms and do not liquefy gelatin. This species ferments dextrose slightly, also levulose and saccharose, and produces a slight increase in the acid content of solutions.

[1] See reference for *Torula communis*.

[2] Geiger, A. Beiträge zur Kenntniss des Sprosspilze ohne Sporenbildung. Cent. Bakt., Abt. 2, 1910, 11.

Pseudomonilia rubescens

The young cells are oval (3 to 5 μ in diameter) in the beginning with filamentous mycelium. The scums are quite thick with more or less marked red color. The giant colonies possess a faint red color. This yeast ferments dextrose in an active manner. It is very sensitive to the action of lactic or tartaric acid.

Pseudomonilia mesenterica

The cells are round (3 to 6 μ in diameter) and often elongated. Old cells contain numerous fat droplets. The scum is thick and strongly folded. The giant colonies grow rapidly and are abundant. This species ferments levulose.

Pseudomonilia cartilaginosa

The cells are oval, from 5 to 6 μ in diameter, pointed at both ends and enclosing crystals in the vacuoles. They are intermingled in a filamentous mycelium with cross walls. The walls of the cells are mucilaginous. The scum possesses a cartilaginous appearance. The giant colonies have a verrucose aspect and the gelatin is quite rapidly liquefied. This species ferments saccharose, but scarcely acts on dextrose and levulose.

MONILIA VINI. Osterwalder [1]

This yeast possesses a very active fermenting function and acts like a bottom yeast. It causes a more active fermentation than the other *Monilia* that are known. It does not stop developing in the presence of very large amounts of acid in the medium in which it is growing. It develops well in solutions with 4 per cent of alcohol, especially in wine. It forms secondary products such as volatile and malic acids. *Mycoderma vini* possesses a less active fermenting ability than ordinary wine yeasts. It has, however, a favorable influence on the wine but produces a secondary fermentation of sugar which has not been transformed by other yeasts in the primary fermentation. It does not contribute a bad or disagreeable taste to the wine.

It ferments dextrose and levulose especially, and saccharose, lactose and galactose less actively. Maltose is very feebly attacked. It seems to possess a soluble sucrase which distinguishes it from *Monilia candida*. The giant colonies on gelatin exhibit borders with well-developed fringes. In liquid media the species vegetates at first

[1] Osterwalder, A. Ein neue Gärungsmonilia, Monilia vini, n. sp. Cent. Bakt. Abt. II. 1912.

in the form of a sediment and later produces flocs in the liquid and a scum like that of a mold. The sediment consists of yeasts about six microns in length. The flocs and scum are made up of a typical mycelium with numerous yeasts.

PARENDOMYCES PULMONALIS. Plaut

This yeast was isolated by Mantner [1] from sputum of a little girl attacked by bronchitis. In the sputum it exists in the form of filaments and conidial yeasts. In cultures the fungus does not give these yeast structures, except there is abundant formation of mycelia. The fungus seems very closely related to *Monilia candida*. Plaut has made a special genus related to *Endomyces*, the genus *Parendomyces*.

Senez [2] described a fungus very much like *Endomyces albicans* which was isolated from the lungs of a patient believed to be suffering from tuberculosis. White granules composed of fine filaments were observed in the lesions. When these granules were inoculated into media they developed into creamy white patches. Carbohydrate media and carrot slants seemed to be best adapted for growth. In liquid cultures there was abundant growth provided the media were not acid in reaction. The organism was a strict aerobe. Both round and filamentous forms were reported. The round cells multiplied by budding. The buds were able to break off and reunited end to end to form filaments. Ascs appeared in old cultures on gelatin. They were large and oval in shape and about 10 μ in diameter. Senez distinguished this fungus from *Endomyces albicans* by the appearance of the lesions in the lungs, its dislike for acid media, the ease of cultivation on alkaline media, the difference in appearance on solid media and its almost negative pathogenicity when demonstrated experimentally.

[1] Mantner, H. Parendomyces pulmonalis, Plaut eine bisher nicht beschriebene Monilia-art. Cent. Bakt. Abt. I, 74 (1916), 203.

[2] Senez, A. El Endomyces pulmonalis. Boletin del Labor. de Bacter. de Tucuman (Rep. Argentina) 1 (1918), 58–80. Bull. Past. Inst. 17 (1919), 636.

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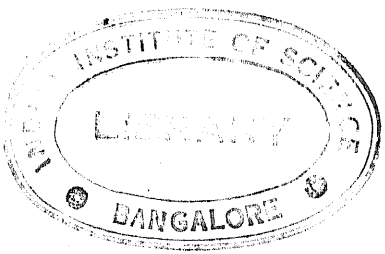
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